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Assistant Commissioner for Patents
Washington, D.C. 20231

On 11-22-02

TOWNSEND and TOWNSEND and CREW LLP

By:

PATENT
Attorney Docket No. 015280-347100US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Robert F. Bonner, et al.

Application No.: 09/456,042

Filed: December 6, 1999

For: DESIGNS FOR NON-CONTACT LASER CAPTURE MICRODISSECTION

Examiner: G. Gablel

Art Unit: 1641

DECLARATION OF DR. ROBERT BONNER

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Dr. Robert Bonner, the declarant deposes and says:

I am a scientist employed at the National Institutes of Health. A copy of my curriculum vitae is attached hereto as Exhibit 1. I, along with Dr. Seth Goldstein, Dr. Paul B. Smith and Dr. Thomas Pohida, are the inventors of the above entitled patent application entitled Precision Laser Capture Microdissection Utilizing Short Pulse Length., originally filed as Provisional Patent Application 60/094,871 on July 30, 1998 and now US Patent 6,420,132.

In the above-entitled patent application, all claims are now limited to that technique known as "noncontact laser capture microdissection." I lodge this Declaration

under 37 CFR §131 to establish our invention entitled Noncontact Laser Capture Microdissection before October 1, 1997. This Declaration is also lodged to establish our due diligence in filing the above-entitled patent application on July 31, 1998. Specifically, Baer et al. United States Patent No. 5,985,085, issued Nov. 16, 1999 entitled Method of Manufacturing Consumable for Laser Capture Microdissection (based on Application 08/984,983, filed December 4, 1997 and claiming priority from a Provisional Application 60/060,732, filed October 1, 1997). This Baer et al. '085 patent describes, but does not claim, noncontact laser capture microdissection. It is the purpose of this Declaration to set forth that as early as January 7, 1997, I observed this phenomenon, and communicated this observation to Thomas Baer. The purpose of my communication to Thomas Baer was to enable early commercialization of this important invention. It is my belief that through this communication, Thomas Baer placed the description of noncontact laser capture microdissection into the Baer et al. '085 patent.

Together with Dr. Lance Liotta, I am one of the pioneers of that dissection technique known as Laser Capture Microdissection (hereinafter LCM). Simply stated, modern biology and medical techniques, especially as related to cancer research, require dissection of specimens literally down to the molecular contents of individual cells in order that a disease (cancer) can be properly diagnosed and treated. As is well known in the medical community, cancer cells are contained in and scattered throughout other cell material. Mechanical dissection is generally unsuitable in such scattered cellular samples. The scattered cellular samples resulted in the development of LCM.

In LCM, a specimen (such as a prostate biopsy) is contacted with a film, the film being chosen for selective activation by laser energy. The specimen is typically visualized through the film, activated immediately overlying a selected portion of the sample for dissection, and adhered to the sample at the "activated" portion of the film. When the film is removed, the adhered portion of the sample attached to the film is likewise removed. "Laser capture microdissection" results. An exemplary patent obtained under this protocol is Liotta et al., U.S. Patent 5,843,657 entitled Isolation of

Cellular Material Under Microscopic Visualization, filed October 10, 1995 as application Serial Number 08/544388. I am a named co-inventor on that application.

On or about the time that LCM was conceived, it was understood to be a technique having great promise. As a consequence, on Tuesday May 14, 1996, the LCM technique was schematically demonstrated to a subcommittee of the House Appropriations Committee and later to the then Vice President of the United States, Hon. Albert Gore. During those demonstrations, questions were specifically directed to the NIH about procuring rapid commercialization of this new technique. The House Committee was assured by NIH officials that all efforts would be undertaken to effect such immediate commercialization.

In order to keep the promise made to the congressional subcommittee, NIH needed to enter into a relationship with a commercial partner. Recognizing the need for a commercial partner, I contacted a number of parties including Mr. Thomas Baer, who subsequently became the founder of Arcturus Engineering, the present non-exclusive licensee of NIH of the LCM patents. My contacts with Mr. Thomas Baer occurred earlier than August 29, 1996. I know this by refreshing my recollection from my log of the e-mail records, which I have reproduced and attached to this declaration has Exhibit 2.

Because of the urgent priority to commercialize LCM, the National Institutes of Health entered into an agreement called a CRADA (Cooperative Research and Development Agreement) with Arcturus. The idea behind this agreement was to allow researchers at NIH and engineers at Arcturus Engineering to freely exchange information.

Beginning as early as August 31, 1996, confidential manuscripts and information were shared with Arcturus Engineering by NIH under a Confidential Disclosure Agreement. (See for example pp 1 and 2 of Exhibit 2).

On or about September 17, 1996, preparations were made with Thomas Baer to enter into a CRADA with NIH. (See for example pp 10 of Exhibit 2). On or about October 1, 1996, a Statement of Work was developed for what now had become known as the Arcturus CRADA. (Page 21 of Exhibit 2).

Prior to any discussions with Tom Baer/Arcturus Engineering, we had developed practical prototypes for harvesting small clusters of cancer cells from stained histology sections and had developed the concept of laser diode activation of EVA polymer films into which naphthalocyanine dyes had been solubilized. These early functional prototypes were displayed to the House Subcommittee on Appropriations during their visit to NIH on May 14, 1996. In order to develop an efficient path to rapid commercialization, it was my decision that it was necessary to place a high priority on understanding the physics of polymer activation by short laser pulses and the nature of the bond and capturing of the targeted tissue cells. Throughout the summer of 1996 at NIH, we worked on developing robust instrumentation, manufacture of prototype dyed polymer films, protocols for sample preparation (e.g., dehydration and desiccation) with an expanding but limited knowledge of the details of the physical processes involved in reliable LCM capture. Two competing major concerns for LCM reliability were the ability to reliably bond and remove target cell clusters from frozen and paraffin-embedded tissue sections and the reduction in inadvertently picking up untargeted cellular debris from the surface of the tissue section. During the summer of 1996, we hired as a consultant Dr. Eugene Goldberg, a polymer adhesion expert at University of Florida, to review our physical concepts and observations and suggest methods for physical characterization of the polymer activation/capture process. On or about December 1, 1996, with a new prototype laser diode microscope we had designed and assembled at the NIH (epi-illumination in an Olympus BX upright), I performed short-pulse activation of a 100μm-thick, near-IR-absorbing polymer film. I discovered that the activated polymer film focally expanded and made contact and bonded with targets as small as a cell nucleus. The target nuclei prior to firing of the laser were positioned up to 17μm below the bottom surface of the polymer film. I immediately realized that this was a critical advance for routine use of LCM in research and, eventually, in clinical diagnostics, as it provided a means of targeting the smallest optical targets without fear of background contamination by untargeted material caused by placing large areas of unactivated film in

contact with the friable tissue section. As this new NIH invention was potentially critical to rapid commercialization of a more robust form of LCM, I disclosed this information in a confidential disclosure to Tom Baer so that he might consider this as an alternative path of commercial development to his early flat-rigid-cap concept. This message specifically discussed the role that an outside consultant could or should play in rapidly determining the nature of the polymer expansion and rapid focal bonding and unproven concepts associated with the polymer physics of film being activated by laser in the noncontact LCM process. (See e-mail beginning on page 48 and concluding on page 50 of Exhibit 2). Since Arcturus and NIH had signed a Letter of Intent to CRADA in November 1996, my question to Thomas Baer was whether the outside adhesion consultant, Dr. Eugene Goldberg, who NIH had hired the previous summer, should be part of a focused discussion on noncontact LCM. On or about this time, it was my understanding that Arcturus had likewise hired a materials consultant.

In the spring and summer of 1996, I first noticed that phenomenon using our CO_2 laser prototype which later became known as noncontact laser capture microdissection. Frequently, individual laser pulses were not sufficient to cause the slightly non-uniform polymer film surface to locally contact, "wet", and bond to the target, but successive pulses did. The initial bonding after several pulses (when one pulse was insufficient) was typically not in the expected center of the beam but at less heated sites at which the polymer tissue surfaces were separated the least. Many such observations remained incompletely understood until the seminal experiments with the prototype laser diode upright microscope and a cylindrical pressure plate were performed in the winter of 1996. In these later experiments, it was possible to precisely select a polymer-film-to-tissue-surface separation gap, and it was demonstrated that the polymer could expand focally ~17% of its thickness and provide the focal pressure to bond a small target cell or nucleus in a millisecond or less. This technique was developed to prevent a phenomenon known as nonspecific transfer in LCM and to allow individual collection and concentration of rare cells within one or more large tissue sections.

In conventional LCM, a film was contacted with the specimen to be dissected. Sometimes particles of the specimen would stick to the film around the adhesion area. The particles so sticking were undesired particles and degraded the LCM process. It became important to control this nonspecific transfer.

Better understanding of some details of the polymer physics surrounding this phenomenon could lead to improved LCM reliability and macromolecular quantitation from pure populations of targeted cells. Beginning in the summer of 1996, Dr. Eugene Goldberg and John Peterson (a chemist in our NIH group) were consulted on polymer surface properties that might facilitate LCM capture while minimizing nonspecific pick-up of cellular debris on the LCM polymer (e.g., by physical or chemical treatment of the polymer or tissue surfaces).

By December 1996 NIH had signed a letter of Intent to CRADA with Arcturus and had drafted a research plan in which my group at the NIH would rapidly develop the first prototypes of small-target, short-pulsed near-IR laser diode LCM and use them to better define the most robust forms of the LCM invention suitable for rapid commercialization. In this CRADA Arcturus Engineering would be responsible for very rapid production of 5 or more commercial beta versions, followed shortly by the commercial sale of LCM, all within a year. By December 1996 we had, in fact, designed and built and were evaluating this first prototype when we completed the fundamental discovery and invention of noncontact LCM. At this time, I wanted Arcturus Engineering to consider this noncontact LCM version for commercialization rather than their thin-film-on-a-rigid-flat-cap concept first discussed a couple of months earlier. In December 1996, all LCM polymer films were being made in my lab at NIH. I had contracted with two companies (PolyVel and Electroseal in New Jersey) to compound a large batch of the dyed EVA polymer and extrude it as a smooth film, but supplying a reliable commercial disposable film format was to be a CRADA obligation of Arcturus. In this context, my confidential email to Thomas Baer (Exhibit 2 at page 60 - 61, the text of my January 7,

1997) revealed critical technical questions that our polymer consultant might be able to address to lead to a rapid decision regarding the optimal format for commercial versions of Noncontact LCM. This e-mail was addressed to Thomas Baer. Attention is directed to item 5) appearing on page 61 of Exhibit 2. For the convenience of the reader, that item 5) is repeated here:

> 5) Presently we have had non-contact transfer - i.e., when the 100um thick film and the tissues section are separated by up to 17um (and the film is below the tissue), the melted film still makes contact with the tissues/wets it/ and then solidifies to form a strong focal bond with it (and the film forms a surface pedestal with the tissue on it). Is this to the volume expansion of the eva on the face transition from solid to liquid? What is the range of fractional volume changes of eva's on melting? What role might surface tension (with the wetted tissue) play in creating the pedestal in the film as it recools?

At the time of this January 7, 1997 e-mail, the full parameters relating to noncontact LCM were not fully understood. During the spring and summer of 1997, we conducted in-lab experiments directed to the expansion of film as it is activated by laser energy. Specifically, I worked on the problem in the spring of 1997. Further, in the summer of 1997, NIH assigned to my research group various summer interns. I had these summer interns conduct further research on this expansion problem. Enclosed as Exhibit 3 are various graphs and pictures of activated film which I have recovered from my computer files.

From the summer and through the fall of 1997, the various parameters appearing in the above-entitled application were developed by the named inventive team.

As previously noted, on or about October 1, 1997, Thomas Baer and Arcturus

Engineering filed Provisional Patent Application No. 60/060,732. This application was perfected as Non-Provisional Patent Application No. 08/984,983, filed December 4, 1997 and entitled Method of Manufacturing Consumable for Laser Capture Microdissection, now U.S. Patent 5,985,085, issued Nov. 16, 1999. It is further noted that they did not claim noncontact microdissection as their invention.

Our experimentation continued with noncontact laser capture microdissection through the beginning of December, 1997. During the period extending from summer 1997 to winter 1997, many of the parameters appearing in the above-entitled United States Patent Application were developed. Beginning in December 1997 and concluding on December 23, 1997, I authored the invention report attached to this Declaration as Exhibit 4.

From January 1998 until July 1998, my invention report was processed by the Office of Technology Transfer, National Institutes of Health. A Work Order was forwarded for the preparation of the above-entitled patent application in early July 1998. That work order was received in July 1998 by the assigned attorneys and the patent application initially referred to in the first paragraph of this document and entitled Precision Laser Capture Microdissection Utilizing Short Pulse Length was filed on July 30, 1998 as a Provisional Patent Application. This application was filed with what I believe is due diligence as I was a participant in that process. That Provisional Patent Application 60/094,871 became Patent Cooperation Treaty application PCT/US 99/17150 filed July 28, 1999. Pursuant to the provisions of the Patent Cooperation Treaty, this domestic patent application was filed January 31, 2000.

Further declarant sayeth not.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title

18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Respectfully submitted,

Dated: November 6, 2002

Robert Bonner, Ph.D.

SF 1306232 v1

Subscribed and sworn to before me, in my presence, this 6 day of November, 2002, a Notary Public in and for the State of Maryland

Notary Public

My commission expires: July 7, 2005

AYUSHA JONES
NOTARY PUBLIC STATE OF MARYLAND
My Commission Expires July 7, 05

EXHIBIT 1

Dale D. Berkley, J.D., Ph.D.

Education:
J.D., George Mason University School of Law, 1996.
Ph.D. Physics, University of Minnesota-Minneapolis, 1989.
M.S. Electrical Engineering, University of Missouri-Columbia, 1982.
B.S. Physics, University of Missouri-Columbia, 1980.

Recent Continuing Education:
Patent Resources Group, "Software Patents, Procuring and Enforcing," Washington, D.C., August 12-14, 2001.

Practicing Law Institute, "Advanced Licensing Agreements for the New Economy," PLI, New York, NY, March 5-6, 2001.

Executive Education Program, "New Product Development & Launch," The Wharton School, University of Pennsylvania, October 10-15, 1999.

Bar Membership:
Virginia State Bar, June 1997.
District of Columbia Bar, April 1999.
Registered U.S. Patent Attorney, Reg. No. 42,319.

Experience:
September 10, 2000 to the present
Technology Licensing Officer
National Institutes of Health (NIH), Bethesda, MD

- Sole responsibility for patenting and licensing medical/research devices and software for NIH inventions, which includes a docket of nearly 300 active invention disclosures and related patents.
- Drafting and negotiating complex licenses involving government owned inventions. Negotiating and drafting exclusive and non-exclusive commercial licenses, evaluation licenses and biological material and software licenses.
- Management of infringement, interference and inventorship matters with respect to NIH hardware and software patents.
- Providing expert advice to NIH scientists and inventors regarding patenting and licensing matters.

September 1, 1999 to September 10, 2000
Patent Administrator and Licensing Officer
National Institute of Standards & Technology (NIST), Gaithersburg, MD

- Responsible for hiring and supervising outside patent counsel prosecuting NIST patent applications.
- Directed infringement analysis of Advanced Encryption Standard candidates and provided IP expertise support for other high profile IP matters at NIST.
- Substantial contribution to new NIST web site for technology transfer office.
- Initiated extensive review of patent procurement process, docketing and

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BERKLEYPUP@AOL.COM

administrative procedures.

- Oversaw administrative details involved in managing and developing the NIST patent portfolio.
- Provided expert advice regarding NIST patent and licensing processes and intellectual property matters to NIST scientists and staff.

March 13, 1998 to the present
Licensing Officer
National Institute of Standards & Technology (NIST), Gaithersburg, MD

- Sole responsibility for evaluating invention disclosures from NIST scientists in the Physics Laboratory (Gaithersburg and Boulder labs), Materials Science and Engineering Laboratory and the Manufacturing Engineering Laboratory.
- Drafted and negotiated complex licenses involving government owned inventions. Negotiated and drafted Guest Researcher, Material Transfer, Confidentiality and other agreements.
- Performed commercial assessment of each invention and reviewed the work of outside counsel. Provided counsel to NIST scientists regarding patent and licensing procedures at NIST.

March 1997 to March 1998
Associate Attorney
Nikaido, Marmelstein, Murray & Oram, Washington, D.C.

- Associate Attorney prosecuting patents in the electrical and other arts, preparation and filing of new utility and design patent applications, preparing infringement opinions and Fastener Quality Act and copyright applications.

November 1995 to March 1997
Law Clerk
Nikaido, Marmelstein, Murray & Oram, Washington, D.C.

- Performed legal research involving patent, trademark and copyright issues of immediate interest to the partners and associates at the firm, involved in patent prosecution since November, 1996.

June 1995 to November 1995
Patent Examiner, Art Unit 2614 (Pulse & Digital Communications)
U.S. Patent & Trademark Office, Arlington, Virginia.

- Examined patents in Art Unit 2614 (Pulse and Digital Communications including spread spectrum and cell phone applications).

June through August, 1994
Summer Intern
Office of the General Counsel, USAfrica Airways, Inc., Reston, Virginia,

- Assisted the General Counsel with business and corporate indemnification agreements for directors, preparation of proxy, directors' questionnaire and other business law matters.
- Helped draft restated certificate of incorporation, researched stock option plans, reviewed countless contracts for services, materials and leases.

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January 1990 through July 1992

National Research Council Post-Doctoral Fellow, tenured at the Naval Research Laboratory, Washington, D.C.

- Preparation of high T_c thin films, especially $YBa_2Cu_3O_{7-x}$, by Molecular Beam Epitaxy and characterization of transport properties to 4K.
- Surface studies of metal overlayers on YBCO films using XPS and Auger analysis.
- High pressure studies to 70 kbar and T>50K of thallium based high T_c single crystals in diamond anvil cell.

1985-1989

Research Assistant, Physics Dept., University of Minnesota,

- Development of techniques for preparation of YBaCuO thin films by electron-beam and thermal source co-evaporation using distilled ozone in Ultra High Vacuum environment.
- Electron-tunneling junction fabrication and characterization using YBCO films to T=2K using SQUID detection scheme.
- La_3S_x thin film preparation and characterization including Hall effect studies.
- HoMoS thin film preparation by electron-beam evaporation and characterization.
- X-ray diffraction bulk studies and surface studies for all films using Auger and X-ray Photoelectron Spectroscopy.

1983-1985

Teaching Assistant (physics and electronics), Physics Dept., University of Minnesota

1981-1983

Assistant Research Scientist

Dalton Research Center, Electronics Design Group, University of Missouri

- Design and development of electronic portable ultrasonic blood flow meter (pulsed 10Mhz acoustic probe) and automatic blood flow occluder for cardiac physiology studies in canines.
- Development and study of ultrasonic probe to determine amplitude of vibrations of ultrasonic transducers used for in-situ ultrasonic imaging.

1980-1982

E.E. Master's Degree Candidate

- Integrated circuit processing techniques. Fabrication of Surface Acoustic Wave (SAW) transducers using 10um line width photolithography for investigation of temperature coefficient of delay of delay lines using various rotated Y cuts of quartz.

1980-1981

Teaching Assistant (electronics laboratory), Dept. of Electrical Engineering, University of Missouri.

Honors: Department of Commerce "Cash-in-a-Flash" Recognition for IP analysis of NIST Advanced Encryption Standard for use in secure electronic and internet

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BERKLEYPUP@AOL.COM

transactions.
Department of Commerce "Cash-in-a-Flash" Recognition for improvement of patent administration procedures in the Office of Technology Partnerships.
Department of Commerce "Cash-in-a-Flash" Recognition for work on development of new Office of Technology Partnerships web site.
Quarter-Finalist, George Mason University School of Law Moot-Court Competition, 1994.
Aneesur Rahman Prize for Excellence in Research, University of Minnesota, 1989.
Gregory Graduate Fellowship, University of Missouri, 1981.
O.M. Stewart Physics Scholarship, University of Missouri, 1976-1980.
University Scholar, University of Missouri, 1976-1979.

Activities:

Invited Speaker: U.S.-Egypt Technology Commercialization Workshop, Cairo, Egypt, May 17-20, 1999.
Invited Speaker: International Superconductivity Symposium '90, Sendai, Japan, November 6-9, 1990.
NATO Advance Study Institute participant, Applications of Superconductivity, Fort Collins, CO, September 14-20, 1990.
Referee: Thin Solid Films, Physica C, Applied Physics Letter, Physical Review Letters, J. Applied Physics.

Societies: American Intellectual Property Law Association, Government Patent Lawyers Association, Virginia Bar Association, District of Columbia Bar Association, Licensing Executives Society.

Patent: Preparation of Superconducting Oxide Films by Reactive Evaporation Using Ozone, A.M. Goldman, D.D. Berkley, B.R. Johnson, U.S. Pat. No. 5,039,657, issued August 13, 1991.

Publications:

1. Pressure Dependence of the Superconducting Transition Temperature in Single Crystals of $Tl_2Ba_2Ca_2Cu_3O_{10-}$, D.D. Berkley, E.F. Skelton, N.E. Moulton, M.S. Osofsky, W.T. Lechter, V.M. Browning, and D.H. Liebenberg, Phys. Rev. B47, 5524 (1993).

2. Surface Characterization of $YBa_2Cu_3O_{7-x}$ Thin Films Supporting Metallic and Insulating Overlayers, D.D. Berkley, P.R. Broussard, and A.M. Ervin, IEEE Trans. Mags., MAG-27, 966 (1991).

3. In-Situ Preparation of High T_c Thin Films by Co-Evaporation Using Ozone Vapor Oxidation, D.D. Berkley, Proceedings of the International Superconductivity Symposium 90, Sendai, Japan.

4. Techniques for the Growth of Superconducting Oxide Thin Films Using Pure Ozone Vapor, D.D. Berkley, A.M. Goldman, B.R. Johnson, Rev. Sci. Inst. 60, 3769 (1989).

5. In -Situ Formation of Superconducting $YBa_2Cu_3O_{7-x}$ Thins Films Using Pure Ozone Vapor Oxidation, D.D. Berkley, B.R. Johnson, N. Anand, K.M.

Beauchamp, L.E. Conroy, A.M. Goldman, J. Maps, K. Mauersberger, M.L. Mecartney, J. Morton, M. Tuominen, Y-J Zhang, Appl. Phys. Lett. 53, 1973 (1988).

6. Ozone Processing of MBE Grown $YBa_2Cu_3O_{7-x}$ Films, D.D. Berkley, B.R. Johnson, N. Anand, K.M. Beauchamp, L.E. Conroy, A.M. Goldman, J. Maps, K. Mauersberger, M.L. Mecartney, J. Morton, M. Tuominen, Y-J. Zhang, IEEE Trans. Mag. 25, 2522 (1988).

7. Preparation of $Y_2Ba_4Cu_8O_{20-x}$ Thin Films by Thermal Coevaporation, D.D. Berkley, D.H. Kim, B.R. Johnson, A.M. Goldman, Appl. Phys. Lett. 53, 708 (1988).

8. Vapor Deposited Superconducting Lanthanum Sulfide Films, D.D. Berkley, J.H. Kang, J. Maps, J-C. Wan, A.M. Goldman, Thin Solid Films 156, 271 (1988).

9. Electronic Structure Changes and Superconductivity in $YBa_2Cu_3O_{7-x}$, D.H. Kim, D.D. Berkley, A.M. Goldman, and R.K. Schulze, Phys. Rev. B37, 9745 (1988).

10. Superconducting Transition in Thin Films of Lead Telluride Doped with Thallium, J.H. Kang, D.D. Berkley, H.M. Jaeger, A.M. Goldman and Dale L. Partin, Phys. Rev. B36, 2280 (1987).

11. Superconductivity in the Ferromagnetic Phase of Polycrystalline $HoMo_6S_8$ Films, J. Maps, D.D. Berkley, J.H. Kang and A.M. Goldman, Phys. Rev. B35, 38 (1986).

Lectures & Symposia:

1. Vapor Deposited Superconducting Lanthanum Sulfide Films, March Meeting of the American Physical Society, March 16-20, 1987, New York, NY.

2. Ozone Processing of MBE Grown $YBa_2Cu_3O_{7-x}$Films, Applied Superconductivity Conference, August 21-25, 1988, San Francisco, CA.

3. In-Situ High Vacuum Preparation of $YBa_2Cu_3O_{7-x}$ Thin Films Using an Ozone Vapor Jet, March Meeting of the American Physical Society, March 20-24, 1989, St. Louis, MO.

4. In-Situ Preparation of High T_c Thin Films by Coevaporation Using Ozone Vapor Oxidation, 3rd International Symposium on Superconductivity, November 6-9, 1990, Sendai, Japan.

5. Pressure Dependence of the Superconducting Transition Temperature in Single Crystals of $Tl_2Ba_2Ca_2Cu_3O_{10-x}$, presented at the National Institute for Standards and Technology, June 19, 1992, Gaithersburg, MD.

6. Government-Industry Partnerships at the National Institute of Standards and Technology, presented at the U.S.-Egypt Technology Commercialization Workshop, Cairo, Egypt, May 17-20, 1999.

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BERKLEYPUP@AOL.COM

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BERKLEYPUP@AOL.COM

Banner 3

EXHIBIT 2

became E-070-98/0

COVER SHEET TO HHS-570
(Employee Invention Report)

Inventors: Banner

Invention Title: Non-contact Laser Cornea Microsection

Scientific/Division Director, Please Check One:

1. Apply for Patent ✓
2. Release Rights to Inventor
3. Dedicate to Public

Please check if this is a CRADA-related invention

Scientific/Division Director's Signature

12/21/97
Date

Notes: Attn. John Fanner-Vickers

CONFIDENTIAL

	For Patent Branch Use
PHS Employee Invention Report	E-Number
	U.S.P.A.#
Use plain paper if more space is needed.	U.S. Filing *(date)*

Part I: To Be Completed by the Inventor

Inventors Names, Phone No.

Robert F. Bonner 301-435-1946
Seth R. Goldstein 301 435-1947

1. Give a short descriptive title of your discovery or invention.

   Designs for Non-contact Laser Capture Microdissection

2. Please provide (in non-scientific terms if possible) a one paragraph description of the essence of your discovery or invention and identify the public health need it fills.

   Building on previous disclosures of laser capture microdissection, a refined method in which the transfer polymer film is placed a small but fixed distance from the tissue/cytology slide and the polymer is focally activated in a manner which insures that it makes contact and bonds tissue only in the desired region with no nonspecific transfer due to mechanical contact This refinement utilizes the significant thermal expansion on melting that can be achieved with a number of thermoplastic polymer formulations such as ethylene vinyl acetat Included in this disclosure is a detailed description of the method of formation of a multi-component tape and simple pressure plate which makes noncontact LCM practical. Further noncontact LCM onto a flexible tape is shown to be easily practiced as a means of concentrating targeted specific cells or tissue elements onto a surface and practical transfer to microvolumes 1uL or less in volume. This will greatly aid in the practice of routine, high throughput LCM coupled to molecular analysis necessary for automated clinical diagnostic use.

3. Who contributed to the inv ntion or discovery? Please identify all colleagues who *could* merit co-authorship credit for the associated publication, whether or not you believe them to be "co-inventors."

The concept of noncontact LCM was largely the development of Robert Bonner through observations he made over the initial year and a half of LCM development which he shared with his colleagues: Seth Goldstein, Paul Smith, John Peterson, and Tom Pohida as well as with his CRADA partners at Arcturus Engineering (notably Tom Baer and Dave Head). Seth Goldstein had observed that different thickness of EVA film if formed onto a substrate with steps against a flat surface would contract proportionally to thickness on cooling to form a stepped EVA (thermoplastic polymer) surface. Further Seth Goldstein made a simple cylindrical pressure plate (in October 1996), which Dr. Bonner used in the initial discovery of quantitative noncontact LCM at gap distances up to 25 microns due to the focal expansion on heating of the EVA polymer by the IR laser diode. James Sullivan recently suggested to Dr. Bonner the use of adhesive tape films rather than machining to form a precise ledge in a rigid plastic substrate.

4. Is anyone outside of the Public Health Service aware of your invention or discovery? If so, please identify them and describe the dates and circumstances.

The entire staff of Arcturus Engineering, Inc. (Mountain View, CA) had been made aware of the early work of Dr. Bonner on the magnitude of thermal expansion of EVA of melting and the ability of this expansion to reliably allow it to span gaps between the polymer surface and the target tissue and that this could allow transfer at focally activated sites while avoiding the possibly of a pressure contact of unactivated polymer surface with the tissue surface leading to non-specific transfer. This discussion was necessitated under the CRADA since our primary object was to develop a reliable LCM transfer process in a commercial instrument. Dr. Bonner realized from the time of the first suggestion by Dr. Tom Baer of his design concept that the major problem with the Arcturus design concept of a large, flat rigid substrate to which the polymer was bonded in the manufacturing process would be the nonspecific transfer due to pressurized contact between the film surface and high points of the tissue (Dr. Bonner had observed nonspecific transfer in earlier LCM transfer experiments in the summer of 1996 using a free film which unlike the Arcturus design permitted the specific transfer region to be easily cut out from the larger regions that were not targeted so as to minimize nonspecific transfer and maintain the high specificity of LCM procured material from tissue specimens).

5. Are you aware of any PHS patent applications that are related to your invention or discovery?

Previous PHS LCM patent applications.

6. Please list the most pertinent previous articles, presentations or other public disclosures, made by you or by other researchers, that are related to your invention or discovery. Also, attach copies. ***please!***

No public disclosures. Although we have published two papers in Science on LCM we have been careful to avoid mention of the volumetric expansion of the EVA which allow it to cross small air (or fluid) gaps and fill the tissue voids when it is focally heated.

7. Please indicate any future dates on which you will publish articles or make *any* presentations related to your invention or discovery.

There are many such presentations planned as well as LCM training offered by Dr. Bonner and NIH staff to numerous outside investigators. Arcturus staff is also training purchasers of LCM in its art. A manuscript is about to be submitted on the details of thermal transients within the EVA polymer film in laser capture microdissection (Applied Optics).

8. In one paragraph, please speculate (and be creative!) about possible commercial uses of your invention or discovery.

This is the essential refinement of LCM as it is applied to smaller targets and the necessity arises for minimizing and random contamination. Further the means of concentrating a number of small individual targets onto a precise region of the polymer film and then reliably sealing these samples within a microvolume for extraction and molecular analysis will be critical for routine diagnostic use of LCM in molecular diagnosis of a large variety of clinical pathologies.

9. a. Is the subject matter of your invention related to a PHS CRADA (Cooperative Research and Development Agreement) involving your laboratory or ICD?

Yes

b. Is the subject matter based on research materials that you obtained from some other laboratory?

No

10. What companies or academic research groups are conducting similar research (if you know)? Can you identify any companies that may be good licensing prospects?

Arcturus Engineering, Inc. (Mountain View, CA) our CRADA partner is definitely interested in licensing this approach. A number of other companies has expressed interest in LCM.

11. What further research would be necessary for commercialization of your invention? Generally, what are your future research plans for the invention and/or for research in areas related to the invention?

Development of commercial film manufacturing techniques to the required precision would have to be developed. This is certainly in the realm of many tape manufacturers who make precision multilayer adhesive tapes.

12. Human Subject Certification: Does this invention rely upon data involving human subjects as defined in and regulated under 45 CFR Part 46?

No

13. Inventor Information:

Name	Degree	Social Security No. (optional)
Robert F. Bonner	Ph.D.	450-90-2494
Position Title: Physicist (section Chief)	Office address: NIH, Bldg.13 Room 3N17, Bethesda MD 20892-5766	
Office Phone No.: 301-435-1946	Citizenship: U.S.	
Home address: 1409 Ingraham Street NW, Washington DC 20011		

Affiliation

x ICD *(specify ICD and applicable box below)* LIMB/NICHD

x GS _ CO _ Special Volunteer

_ GM _ Visiting Fellow _ Other *(specify):*

_ SES _ Visiting Associate

_ Non-ICD Affiliation *(specify):*

What specific personal contribution did she/he make to this work?

Development of principles involved, experimentation, and integration of the necessary concepts into the desired refinement. Dr. Bonner originated the concept of laser capture microdissection as presently practiced. He further synthesized the current concept based on his own work and observations and the assistance of Dr Goldstein (see below) and with the suggestion of James Sullivan on the use of tapes rather than machining to make precise steps (25-100 microns high) onto a rigid surface.

Name Seth R. Goldstein		Degree Sc.D.	Social Security No. *(optional)*
Position Title Biomedical Engineer		Office address NIH, Bldg.13 Room 3N17, Bethesda MD 20892-5766	
Office Phone No. 301-435-1947	FAX No. 301-496-6608	Citizenship x U.S. _ Other:	
Home address	7103 Laverock Lane, Bethesda MD 20817, Montgomery County		

Affiliation
x ICD *(specify ICD and applicable box below)* ORS/BEIP

x GS _ CO _ Visiting Scientist _ Special Volunteer
_ GM _ Visiting Fellow _ Howard Hughes
_ SES _ Visiting Associate Fellow
_ Guest Researcher

_ Non-ICD Affiliation *(specify)*:

What specific personal contribution did she/he make to this work?

Development of films and cylindrical pressure plates which enabled early experimentation that led to this integrated refinement concept. Originated concept that different thickness of molten EVA on a flat release lined surface would contract proportionally to thickness on cooling to form a stepped EVA (thermoplastic polymer) surface.

14. Inventors' Signatures

_ This report is submitted pursuant to Executive Order 10096 and 10930 and/or Department Regulations. PHS employees have an obligation to report inventions they make while employed by PHS to OTT. Under E.O. 10096 and 367 CFR 501 the Government shall obtain the entire right, title, and interest in inventions: (i) made during working hours; or (ii) with Government facilities, equipment, materials, funds or information; or (iii) which bear a direct relationship or is made in consequence of the official duties of the inventor. If you are employed by PHS to conduct or perform research it is presumed that the invention was made under the foregoing circumstances. If this is not the case you must contact your Technology Development Coordinator (TDC) and provide the TDC with the details pertaining to this particular discovery or invention so that a determination of rights can be made.

Inventors' Signatures	Dates	Witnesses' Signatures	Dates
Robert J. Bo	12/23/97	W-S. Eden	12/23/97
Seth Goldstein	12/23/97		12/23/97

Part II: To be completed by the Technology Development Coordinator.

15. Institute(s) or Agency(s) sponsoring this invention

16. Patent prosecution fees are to be charged to

CAN:	1012		
ICD:	NICHD		
Authorizing Official *(Typed)*	Signature		Date
GORDON GUROFF			12/24/97

Send 3 copies of this form when completed to the OTT Patent Branch.

Noncontact Laser Capture Microdissection and the design and method of manufacture of special tapes and rigid substrates to accomplish noncontact LCM

This describes a method not known in the prior art, of making a polymer surface activated by a focused light beam (e.g., near IR laser diode) so that this surface is recessed by precise distances on the order of 5 to 20 microns or more from a border material which does not form a bond with a tissue or biological preparation surface to which it is pressed. Further this method includes a pressure plate (e.g., cylindrical rod) which places the border zone in direct contact with the tissue surface while holding the activatible polymer surface at a fixed distance from the tissue surface (which is usually greater than the thickness of the tissue specimen but much less than the thickness of the polymer layer). Using previously disclosed LCM concepts and materials, this assembly can be placed onto a region of interest, specific tissue components identified by the microscope and targeted by the laser beam which heats the polymer causing it to expand and make contact and bond (e.g., impregnate fluid or air spaces in the targets and then cool in place form a focally strong bond to the target). We have shown that the standard EVA polymer we have been using for LCM (Dupont ELVAX 410 ethylene vinyl acetate) typically expands by >10% when focally heated from room temperature to its melting point. Thus if the targeted polymer film is melted from top to bottom at a focal spot, it will expand forward as a pedestal roughly 10% of film thickness. Thus a 100 micron thick film when focally melted will expand greater than 10 micron and thus can explore an air gap of this thickness between it and the tissue until it finds the tissue at which point it expands into the void spaces of the tissue forming a strong focal bond. We have observed that the actual distances traveled can be 2-3 fold greater than these values due to higher temperatures reached in the irradiated segment. Heating of the region surrounding the melted laser irradiated plug by radial heat flow might cause the surrounding solid polymer to expand radially onto and constrict the melted zone pushing the polymer pedestal even farther from the original polymer surface. In general when the

polymer cools it remains extended within the tissue until the film is rapidly "peeled" or lifted off the tissue (biological) specimen which tears at the borders of impregnated zone (typically a cylindrical podestal). At this point the polymer and impregnated targeted tissue snaps back towards the original polymer surface. Typically the recoil is greater than 50% of the original extension. Thus with our existing EVA polymer used in LCM, we can activate a 100 micron thick polymer layer to extend 10 microns so as to cross a uniform 5 micron air gap, then impregnate a 5 micron thick tissue slice to which it bonds strongly. On separation from the tissue, the tissue polymer surface retract more than 5 microns so that this region may be placed again onto a different region of the tissue specimen without making contact anywhere except the border zone previously mentioned. Thus repetitive transfer of different spots may be made (by appropriate translation of the film and its substrate) and concentrated (i.e., placed at target to target spacing that are much less than those within the tissue section) in a small central zone on the transfer surface. Once sufficient homogeneous biological targets have been accumulated on this transfor surface, it make be placed on or in a microvessel for extraction and molecular analysis.

A specific refinement of this placement process includes the annular sealing of the thermoplastic film to the open top of a (cylindrical) microchamber in a manner which seals the chamber with the transferred tissue (biological targets) placed at the center of the chamber or the inside surface of the polymer forming the lid. A further specific refinement uses the thermoplastic sealing properties of the EVA film to form this tight seal either by an annular laser source (or spot scanned in a circle) or by a annular heated pressure plate. This later approach is more easily realized if the substrate on which the recessed thermoplastic polymer was originally formed is relatively thin such as a 100-200 micron thick Mylar (polyester) film.

A specific preferred geometry is to manufacture a "nonstick" polymer tape so that its room temperature thickness is larger than that of the desired thermoplastic adhesive

polymer thickness by the amount of the desired recess for the activatible polymer. This can be accomplished by manufacture of the EVA by casting onto the substrate at a higher temperature so that the differential expansion of the EVA and "nonstick" border cause the EVA to form a flat surface at the elevated temperature which on cooling leads to the desired recess. For example, we could form a laminate of 200 micron thick polyester (1 cm wide) with a 200 micron thick strip of polyamide (3 mm wide) on both edges forming a central channel 4 mm thick into which a fine continuous bead (rod) of hot ELVAX 410 (with IR absorbing dye) is extruded and then hot rolled by a smooth drum to form a flat surface which on cooling leads to a 1 cm wide tape with a 4 mm wide central section of ELVAX 410 on polyester which is recessed by 20 microns from the border strips of polyamide bonded to the polyester. Thus we propose a simple scheme for the manufacture of a precision recessed tape for "noncontact LCM" . Note that an alternative is to form the same sort of release surface (polyamide) border on a rigid substrate and then fill the central region with EVA. [In the current manufacture of the Arcturus caps and an annular ring (perhaps with a relief channel for excess EVA polymer) of polyamide could be bonded to the cap and then the central region of this ring filled with melted EVA which on cooling would be precisely recessed.

A further refinement of noncontact LCM uses a previously disclosed design f r periodic marking of the tape so that transfer could be placed periodically in well defined locations. Originally this concept was developed so that the punching out of small transferred regions into extraction and molecular analysis vessels could b performed without a separate optical location of the transfer regions. In its pres nt usage, a "noncontact LCM" tape can be translated a fixed increment relative to periodic indicator markers on it between each set of LCM transfers, where a set of transfers indicates multiple transfers of individual targets which are homogeneous and to be pooled into one sample for molecular analysis. Much smaller separations between the individual LCM transfers within each set of transfers creates a cluster for each set

within a small region (in the example abov it might be within 0.5 mm while different sets might be spaced on 2 mm c nters). If the micro chambers used for molecular extraction and analysis are formed as a linear array of wells (with a diameter slightly greater than the individual transfer clusters or d>0.5 mm in the above example) with exactly the same periodic repeat as tape translation between microtransfer sets (2 mm in the example above), then this scheme allows a large number of sets of transfers to be accumulated onto the continuous tape and then continuously transferred and (heat) sealed onto the linear array of microchambers for molecular extraction and analysis. This greatly increases the efficiency of the current LCM process and provides means to reduce the volume of the molecular analysis systems to such small volume that the analysis may be performed more rapidly, at lower reagent cost, and with greater precision. Further this design or its analogues would offer significant advantages for automation of analysis and tracking of samples over the current LCM transfer caps, particularly when incorporating state of the art microfluidic processing of microvolumes).

SAMPLE CLAIMS:

1) a refinement of laser capture microdissection (LCM) in which the activatible polymer surface is purposely separated from the target tissue by a fixed spacing so as to avoid contact in regions not specifically thermally activated (e.g., by a laser pulse) and thereby avoid nonspecific transfer of random contaminating specimen elements by physical contact (e.g., under pressure at room temperature or slightly above). This refinement can allow more reproducible spot size and dosimetry of transfer by minimizing sample (e.g., target tissue) density and surface irregularities.

2) A series of refinements of noncontact LCM which allow the positioning of the activatible thermoplastic polymer (e.g., EVA) surface at a fixed distance from th tissue surface.

a) laminate a thin "nonsticky" or release-lined polymer film (e.g., a polyamide adhesive tape or polyester, polyur thane, etc.) that is laminated to the substrate (either a flexible tape or rigid pressure plate) so as to form a border nonsticky region that makes sole contact with the specimen (tissue) surface to be transferred (see figure 1). Onto this laminated step surface the thermoplastic polymer (e.g., EVA) is coated at elevated temperature filling the groove between the border zone. For example, on cooling the surface of thermoplastic polymer (e.g., EVA) which has a higher differential coefficient of thermal expansion will become recessed from the border zone material by a distance equal to the [ΔT, the temperature difference on cooling] times [T_{border} . the thickness of the border film] times [the differential coefficient of thermal expansion]. In this manner precise recessed polymer films can be made of any desired thickness and distance of recess by selection of materials with required differential coefficients of thermal expansion.

b) a refinement of a) in which the border zone consists of an annular ring of film (with or without escape channels for excess molten EVA or other chosen thermoplastic polymer) which is laminated to the LCM rigid cap surface (present Arcturus design) or any other rigid pressure plate substrate.

c) a refinement of a) in which the border film is laminated to a strong polymer film in any of a variety of geometries forming a flexible stepped substrate suitable f r continuous roll filling with a molten activatible polymer film which becomes uniformly recessed on cooling.

d) a refinement of c) in which the completed laminated transfer tape is applied to the tissue by a convex pressure plate (e.g. , a cylinder parallel to the specimen slide and raised and lowered during the cycle of targeting, bonding and removal of LCM targeted elements from the specimen).

Although LCM has largely discussed using those specific polymers that have been shown to be practical in these applicati ns, it is understood that there are a large variety of polymers which can effect this noncontact transf r. Further there are a

variety targets besides cells or biological specimens to which LCM can be applied. The currently discussed ability to pick up specific microscopic objects from a heterogeneous or disperse collection and transfer them as concentrated samples of these specific objects (identified under microscopic visualization or its equivalent) to microvolumes of 1 microliter or less may have a variety of uses in nanotechnologies.

3) a further refinement of the tape concept previously disclosed (LCM Tape Handling patent application) in which precise periodic spacing of the centroid transfer regions of a set of concentrated homogeneous targets corresponds to the spacing of an array of microwells on which the LCM transfer strip is precisely placed and sealed to form microchambers. (note these targets might alternatively be purposely chosen to be heterogeneous in some identifiable characteristic so a to more uniformly sample a desired populations of objects or alternatively to mix specific components or reactants within the microchamber)

4) a refinement of 3) in which the sealing is accomplished by an annular pressure plate pressing the activatible surface against the top of the rim of the wells and sealing with either a contact adhesive, an annular heat source, or a scanned laser beam.

NONCONTACT LCM.

Figure 1

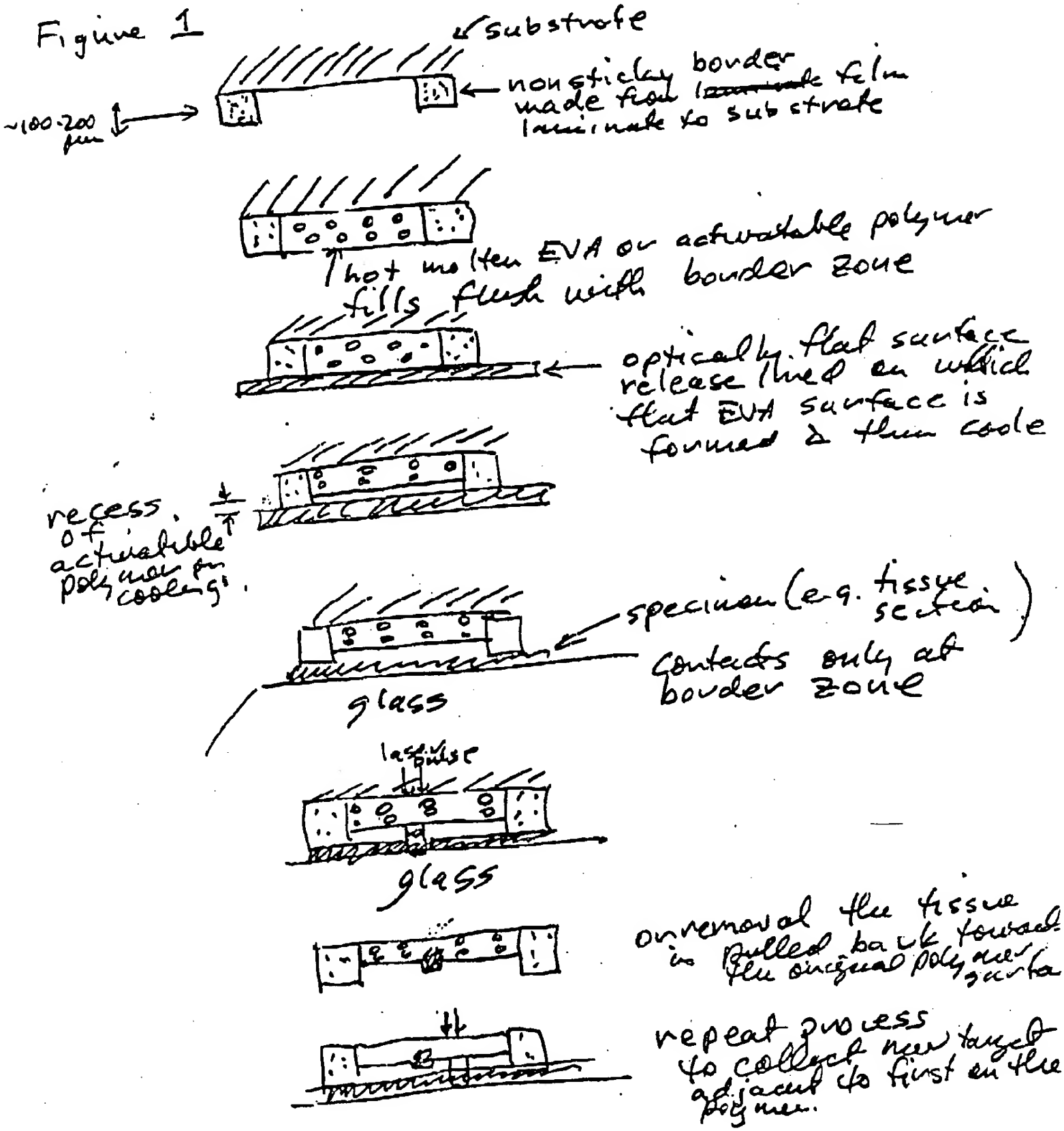
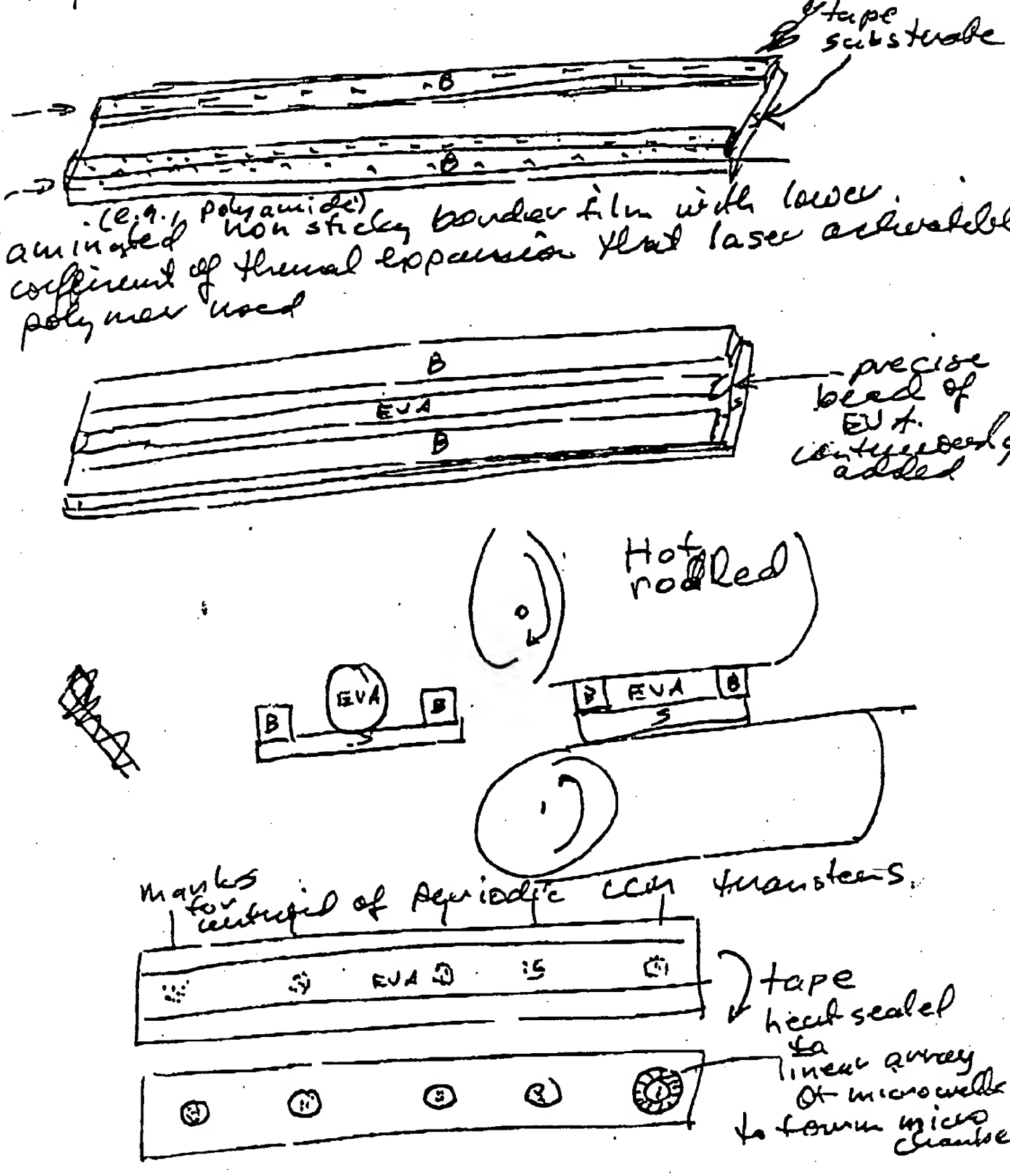
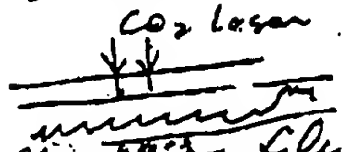


Figure 2 Linear tape



NON Contact LCM

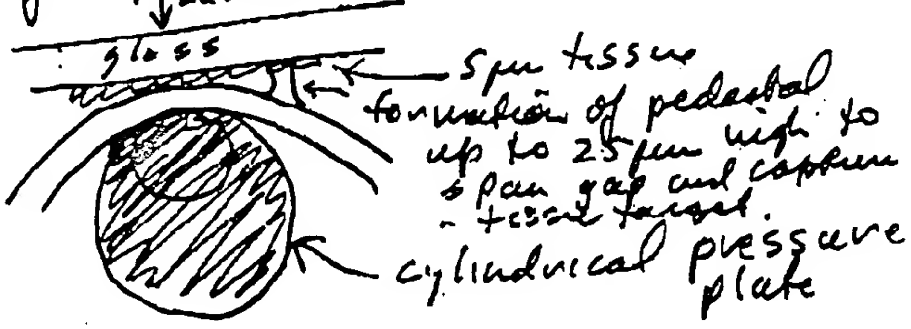
Original discovery



multiple pulses allow fibrin film to bend and wet it. The border of the wet region can then easily be extended by successive pulses [observed as method by R Bonner Spring 1996 and used extensively for tissue targeting in Summer 1997]

original measurement of reproducible spacing noncontact ablating gap.

Sept-October 1996.



CV: Robert F Bonner , Ph.D.

Bldg 13 Rm 3W16, NICHD, NIH, 9000 Rockville Pike, Bethesda, MD 20892-5772
Tel.301-435-1946, FAX:301-435-5035, e-mail:bonner@helix.nih.gov

Education:
1973 Ph.D., Biophysics, Johns Hopkins University, Baltimore, MD
1969 BA, Physics, Rice University, Houston, TX

Employment History:
1997-present Chief, Section on Medical Biophysics, Laboratory of Integrative and Medical Biophysics, NICHD, NIH
1995- 1997 Chief, Biomedical Optics , Biomedical Engineering & Instrum.Program, NCCR, NIH.
1995 Visiting Professor of Physics, Universite de Paris, Paris, France,
1990- 1997 Head, Lasers and Modern Optics Research Facility , NIH
1977- 1997 Senior Physicist, Biomedical Engineering and Instrumentation Program, NCRR, NIH
1974- 1976 Research Fellow, Max Planck Institut biophys. Chemie, Molecular Biology Abt., Goettingen, Germany
1973- 1974 NIH Postdoctoral Fellow, Department of Biophysics, Johns Hopkins University, Baltimore, MD.

Research Interests:
Development of molecular diagnostic technologies and methods using optics , Medical Biophysics, Biomedical optics, laser microsurgery. light-induced damage mechanisms in therapy and pathology, Optical Coherence Microscopy, Laser Doppler Blood Flowmetry, Modeling of complex biophysical problems and data analysis. Random Walk Theory. Photon Migration Theory.

Professional Societies: Biophysical Society, Optical Society of America, American Physical Society

Honors:
1999 Finalist, Discover Technology Innovation Awards, Medical Technology
1996 AAAS Science Innovation Topical Lecturer on "Optical Tomographies"
1992 Best Annual International Research Paper, Societe de Radiologie, France
1989 Lawlor Award , 54th Annual Meeting of the American College of Gastroenterology

Fellows Supervised:
M J Yadlowsky, J. M. Schmitt, A. Knüttel, A. H. Gandjbahhche (DCRT), P. C. Douek, A L Bartorelli (NHLBI), H M Druce (NIAID)

Selected Publications

Books and Conference Proceedings (4 in total)
Bonner RF, Cohen GE, Laue TM, and Priezzhev AV, Editors, Biochemical Diagnostic Instrumentation Proc SPIE 1994; 2136:1-354.

Articles and Reviews (from 97 papers and 7 patents)

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EXHIBIT
1

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EXHIBIT 2

Date	To:	From	Subject	
8/29/96	Thomas Baer <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	Re: Visit to NIH	Dear Tom, Either time would be alright with me. I can't speak for my colleagues in NCI at the moment, but they are not critical if you want to trust me to state the potential magnitude of the applications. I did receive your hard copy and will treat it as duly executed and send you a copy of our Science manuscript. This is strictly confidential. When you come we can discuss what are next prottotype will be, and how we see the instrument developing and being commercialized. Look forward to seeing you soon. Bob >Dear Bob, > >I need to make travel arrangements for my next trip to Washington DC, and I >would like to confirm our plans for a meeting at NIH on Thursday, morning >September 12. As I mentioned in my previous email I could also meet with >you late afternoon on Wednesday, September 11. Could you please let me know >as soon as possible if this dates and times are convenient for you? > >Thanks!!! > >Tom Baer > >P.S. Have you received the hard copy of the confidentiality agreement?
8/31/96	Thomas Baer <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	Re: Visit to NIH	Tom Thursday at 9:30am would be fine. Bldg 13 RM 3N17. My office number is 301-435-1946. I will fax you a copy of our Science manuscript today. It is confidential.

Date	To:	From	Subject	
				Bob >Dear Bob, > >Why don't we plan for Thursday Morning September 12. I can plan to arrive >anytime that is convenient for you. I am looking forward to our meeting. > >Thanks for acknowledging receipt of the confidentiality agreement. I will, >of course, treat the preprint as confidential material. > >Best regards, > >Tom Baer
9/05/96	jimb@amb.niddk.nih.gov Cc: buck	bonner@helix.nih.gov (Robert Bonner)	microdissection for PCR, RT-PCR and enzymes	Jim, I have been developing a technique for routine microdissection of pathology with Lance Liotta and Mike Buck(NCI) that we call laser capture microdissection LCM. We have successfully demonstrated reproducible "quantitative" procurement of targeted tissue from a thin section while viewed by an inverted microscope. Our current transfer resolution is 60 um (diameter) spots and I have shown that any microscopic identifiable tissue such as glomeruli can be specifically transferred onto a film for subsequent analysis by PCR (mutations, infectious agents), RT-PCR (gene expression), and enzyme activity (e.g., matrix metalloproteinase-2). The technique is fundamentally much simpler than manual microdissection techniques (e.g., previously developed by Mike Buck). Though LCM subjects the transferred tissue to brief thermal transients we have had very good results in our subsequent biochemical analysis (PCR, RT-PCR, gelatin zymography). In our initial paper (Science in press) we mention that we can identify

Date	To:	From	Subject	
				and transfer glomuleri in a kidney thin section at a rate of 60 in 6 minutes. Because no manual effort is required, microdissection of specific target pathology in the kidney (presently to 60 um resolution) can be rapidly and tirelessly obtained for studying site specific PCR, RT-PCR and enzyme analysis. I thought that the ease of obtaining pure glomerular tissue for such analyses might be of interest to you in studying kidney disease. I am particularly interested in developing techniques for quantitative analysis of gene expression or enzyme activity in pathologies. If you or any of your associates are interested, Mike Buck and I could discuss the technique in more detail with you. Regards, Bob Bonner
9/06/96	Thomas Baer <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	Re: Upcoming Washington visit	Tom, Presently we are designing a second generation laser microscope which uses a laser diode to heat the film (which has an added strong IR absorber). Although we started this project exactly a year ago, it has generated enormous excitement among key players at NIH (Dr Klausner - NCI director and Dr Collins- NCHGR) and has been demonstrated to members of the House and Senate Appropriations Committees (who loved it). We have broad patent protection pending. I want to make certain that the technique is available to outside investigators and then clinical pathology depatments rapidly in a sophisticated form along the lines we are developing. Klausner will support considerable internal development and has stated to Congress that the technique will be available in their research centers, etc. The biggest logistical problem will be making a rugged, optimal design and making it available rapidly with sufficient technical support to keep running at multiple sites. NIH will likely be the training center and supprot multicenter clinical research with it, but it would be very difficult for me to run a contracting operation to have multiple copies made, shipped and maintained.

Date	To:	From	Subject	
				I am looking for a corporate partner(s) to make the device. We are also talking to Olympus. I want you to consider the ability (and advantages/disadvantages) of a smaller American firm making the laser system as an attachment to commercial pathology microscopes. How quickly it could be done, whether it would be possible to build and support 30 instruments in a 3-6-12 month time frame. We are continuing to develop more sophisticated films for the final laser diode system and this would probably be held separate from the laser microscope licensing. >Dear Bob, > >I have read through the preprint several times and I think the whole concept >is very exciting. I am looking forward to my visit next Thursday at 9:30am, >Bldg 13, RM 3N17. I hope we can discuss how Arcturus can assist your >efforts on this project. > >Is there any thing else that we need to cover before I leave (on Sunday)? > >Best regards, > >Tom Baer
9/06/96	Thomas Baer <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	Re: Upcoming Washington visit	Dear Tom, There are a lot of similarities between our approach of heat activation of the "IR-dye containing" film with a laser diode and recordable CD-ROM technology. If you have knowledge or descriptions of the current state-of-the-art and sources of components for recordable CD-ROM's I would appreciate your bringing it. Regards, Bob

Date	To:	From	Subject	
9/10/96	laserPCR (NIH INTERNAL)	bonner@helix.nih.gov (Robert Bonner)	Meetings: Tom Baer & Olympus	Dear Group, Tursday, September 12 9:30 am: Tom Baer will be visiting Thursday morning (9:30 am) to discuss the possibility of his start-up company manufacturing a laser-diode microscope for laser capture microdissection. Dr Baer has a lot of experience in laser diodes and laser diode optical instrumentation and biomedical devices from a corporate side. He is presently raising investment capital with Milton Chang (well known founder of a number of silicon Valley optical companies) and seeking a "biomedical" product. He is very honest and direct and should be able to give us considerable advice on reasonable ways to proceed. Friday September 13 ~10am: I have just heard from Mr Reinhardt Enders of Olympus America, that both Olympus Japan and Olympus America have signed the Confidential Disclosure Agreement and he and Mr Naito will meet us on Friday morning (~10am). It should be interesting. If you have any ideas on specific points on the agenda please send me an email. Thanks. You are all welcome to attend any of the discussions you wish - please let me know of your interest. Bob
9/11/96	harfordj@od.nci.nih.gov Cc: lance	bonner@helix.nih.gov (Robert Bonner)	meetings with Olympus on LCM	Dr Harford, I wish to inform you that I have arranged for two meetings this week with companies interested in developing the laser microscope for the laser capture microdissection technique that my group in Biomedical Engineering has been developing with the Lab of Pathology, NCI (Liotta). As you may remember this technology was discussed last August 8 in Dr Klausner's office and he said you should be our contact person. All parties: Arcturus Engineering (Dr Tom Baer) and Olympus America (R. Enders) & Olympus Japan (M. Naito) have signed Confidential Disclosure Agreements prepared by Gary Colby. Dr Baer will visit tomorrow morning and the Olympus group on Friday. These are initial discussions to discuss the possibility of these companies making commercial systems for early multicenter trials as

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				well as for commercial markets that we expect will develop. If you would like to give us any guidance or would like to have the opportunity to meet these visitors, please let me know. Thanks.
9/13/96	laserPCR	bonner@helix.nih.gov (Robert Bonner)	Revised invention reports	Hello, I am still working on invention reports for CIP of Lance's patent, but time is close so I am sending these copies for comments. Ithink one CIP patent should be on the laser capture microdissection method/device and the other on the film. Bob
9/14/96	mbuck@helix.nih.gov (Michael R. Emmert-Buck)	bonner@helix.nih.gov (Robert Bonner)	Re: LCM	Mike, As I said directly the Steve Bova interaction would be great. I sent Lance the following update on the Olympus visit yesterday. "Olympus Japan (M Naito, head of their microscope application R & D branch) and Olympus America (R Enders) visited today for 4 hours and discussed possible interactions with the laser capture microdissection. They had signed the Confidential Disclosure Agreement drafted by Gary Colby. I explained to them our desire to develop rapidly several laser diode microscope prototypes and in the longer term to insure that this technology is commercially developed and available. I reviewed the figures in the Science manuscript and demonstrated the carbon dioxide laser microscope and film microtransfer to them. The information I supplied to them was similar to that that I provided Tom Baer of Arcturus the previous day. I suggested to them 3 possible alternative paths to our interaction: 1) we might enter into a CRADA (with Olympus America), in which we would expect considerable resources from Olympus being dedicated to rapid prototype development, 2) a sales engineering approach in which Olympus would suggest existing Olympus components and how they might interact with our laser diode fiber

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				system to meet our specific needs for prototypes (we would purchase all such components), once we assembled such a system Olympus might nonexclusively license the patents and sell a specially configured system, and 3) Olympus could simply wait until a clear market existed, and not be the first to market (presumably licensing the patent when they decided to enter the market). Unlike Tom Baer who seemed very excited and anxious to develop a CRADA, Mr. Naito and Enders appeared guarded about their response or level of enthusiasm. When asked to do so, Mr. Naito rapidly thought up a couple of simple configurations in which the ability to change laser beam sizes in steps could be incorporated with existing components. Their strengths were clearly their understanding of microscope imaging, requirements of pathologists, and the uses of the diverse range of possible configurations of existing Olympus components. Mr. Naito appeared critical of the quality of the microscope images we were obtaining in our desiccated samples using the Olympus CK2 and tried to come up with configurations which would improve image quality which he thought would certainly need to be better for single cell targeting and transfer and might be required to satisfy pathologists when targeting larger groups of cells. Olympus has some experience in developing digital user interfaces and image archiving in a image work station they are developing. It was unclear how flexibly or rapidly they could develop a system, though it was clear that a higher level decision would be required for anything other than a minor effort. Frankly, I got the feeling that they were a little disappointed by the technology and would probably not commit to a significant effort on the project. But they may have viewed their visit as a fact finding mission only, with official responses requiring considerable consensus building back home. Regardless, it was quite a contrast with the open enthusiasm displayed by Tom Baer, the previous day. I personally would hope to receive high level sales engineering advice from Olympus as we decide on a final platform and

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				microscope configuration for our first laser diode microscope system, but expect little else in the short term." Mike, after a day of reflection, I think there were some important messages in the Olympus visit. We have been driven to get the system to work, and have been quite excited by our successes. However, the system is still far from a practical routine research tool in a "normal" environment - I think that is what the Olympus team was seeing. They were rightly concerned about the poor quality of the tissue imaging, and in particular that the difficulty in imaging single cells well would preclude targeted single cell transfer. More importantly the poor images of larger scale structures may be disconcerting to the pathologist so that careful interaction between viewing cover-slipped adjacent sections and the target specimens may be required. This in itself is a time consuming complicated process. Can we improve the tissue imaging so its not necessary or do we provide video reference images of the cover-slipped regions while targeting desiccated slides? We have tried to go cheap and simple, however the low cost camera we are using may not replace the higher resolution direct optical image to the pathologist's satisfaction - therefore we may have to go to more expensive components. We need to have a meeting soon to discuss the best configuration for the first laser diode microscope and what would be required for the first beta-test site microscope at Hopkins. Seems to be a natural to have Steve Bova's interactions. Bob >Hi Bob, > > I was looking for the H/E's from the 5 Chinese esophagus cases we >did for the LCM manuscript... do you have them or are they in the laser >room somewhere? > > Also, I just got off the phone with Steve Bova at Hopkins. He

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				is >still very anxious to become involved in the LCM project, and would like to >come down to NIH to do a small study in the near future. Is that OK with >you? I told him it should be fine, but I would talk to you first. He >knows we are still using the CO2 laser so our resolution is not single cell >yet... apparently he has a study which could be done with 100 um resolution >or so. I also invited him to participate in our next group meeting. >There's a good chance he can get a small extramural technology grant from >NCI to officially join our team as a developmental collaborator. I think >it would be good to have him and Hopkins on board in an official >capacity... should help make LCM the method of choice in the future. > > What do you think? > > Best, > > Mike
9/16/96	Thomas Baer <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	Re: Visit to NIH	Tom, We enjoyed and were excited about your visit. The Olympus visit was very difficult for me to read. I believe they might be useful to give us sales engineering assistance in selecting the right stock components for our first laser diode microscope system. If you are committed to a CRADA with us, I could use your immediate help in getting OptoPower to get a 840nm laser diode for the fully integrated microprocessor controlled fiber optic system we ordered from them recently. The most critical developments needed for a clinical will not be the laser microscope (which we could do here) but rather the refinements

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				of a multilayer film with high near IR absorption and optimal tissue bonding, and one or more improved ways to take the tissue transferred on the film into the set of biochemical reactions and analysis. I expect that Hopkins will be working closely with our groups at NIH starting shortly which will be good to have a set of pathologists looking at our concepts with new eyes. I will talk to you later in the week. Bob
9/17/96	Thomas Baer <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	Re: Visit to NIH	Tom, The Science manuscript you saw was accepted. Lance Liotta is anxious to be making some progress. It is important to firm up your intrest and develop a reasonable STATEMENT OF WORK for the CRADA and appropriate guideposts/timelines that we both feel comfortable with. I need to have a series of conversations with you. Email can be a starting point. I know we can develop the next microscope ourselves - but could do better with your help/advice. We certainly need help with taking a brassboard to a commercial research tool (and later to a commercial clinical tool). I hope you can make a go (investor satisfaction) of first commercialization with a research tool - since the time line for a commercial clinical instrument for pathology departments would seem to be too long for a start-up. We have lots of ideas about the film for the laser diode and a number of contacts. We could probably get something made in a month. I don't know how to evaluate your expertise/contacts in this area and uncertain how we should proceed in this area. We would like to specify a laser diode immediately, but since OptoPower has trouble providing a 840nm LD, I am relooking at other similar dyes. Aldrich has a good one at 808nm (which we have not yet tested). I wonder what specific naphthalocyanine dyes the erasable CD industry uses; why they made a specific choice, and what are the suppliers of those dyes. Should we use their choice and perhaps get a cheaper more reliable first film? I am tracking done my sources, but this is why I

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				called and left the phone message I did. In we are to work together, I would like to discuss this choice dye/ LD wavelength with you before I make it. Of course we could buy multiple laser diodes and try a number of dyes - but this project has incredible time pressure and I want to make the right choice now. Bob >Dear Bob, > >I am still committed to and excited by the possibility of a CRADA with you >and NIH on this project. I have contacted the OTT people and they seem >willing to expedite an agreement. > >I will contact OptoPower and see what I can do to speed things up. > >I would be glad to help on the film development, if you would like input in >this area. I have several contacts that have experience in organic dye >development and plastic film manufacture. I would enjoy collaborating on >this part of the project as well. > >I will be in touch after I call Optopower... > >Best regards, > >Tom Baer
9/17/96	laser	bonner@helix.nih.gov (Robert Bonner)	Re: Opto-Power	Paul, I don't think 830nm is a good choice since it is in a window where we don't have a good dye. I would rather take a flier on 808nm given the new Aldrich dye I found. Perhaps John and I can talk to the Aldrich people tomorrow and maybe touch base with other sources before we

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				make a decision. We can have some answers by noon so that we could talk to Optopower about specifically what they could supply us from current stock. Bob >Bob- > >OptoPower has our 830nm diode available and are planning to send it for >fiber coupling today. I asked if they would hold off until tomorrow so that >we can decide what course of action to take. Apparently, 808nm is not a >problem. It would seem to me that for $1500 or so we can get any wavelength >we choose at a later date. We know that the 830nm is a viable dye at >present, it dissolves, and at NRL appeared to function. Aside from >convenience in having a wavelength readily available, 808nm will not be that >different from 830nm. Whatever we learn at 830 should be directly >applicable to 808. We need to get experience with these diodes, the fiber >coupling and determine how we intend to integrate into the microscope. > >I imagine you are tied up with Judy's poster. Good luck. I for one really >appreciate the effort(s) that you are putting into making Biomedical Optics >a viable entity. > >Let us decide in the morning how we want to proceed. >Paul D. Smith >13-3W16 >5.1945(tel); 6.6608(fax) Wed Sep 18 17:38:10 1996 To: laser

Date	To:	From	Subject	
				From: bonner@helix.nih.gov (Robert Bonner) Subject: Cc: Bcc: X-Attachments: Message-Id: <ae65e27f110210030f63@[165.112.76.4]> I measured the absorption spectra of the dye in E-540 eva that John mixed up today. The #8 (the vanadyl compound I suggested) had a peak at 809nm and an OD of ~0.23 per 100um per 0.1% w/w and a visible OD ~10fold less The #9 (a vanadyl Aldrich phthalocyanine) had a peak at 800nm and an OD there of ~0.64 per 100um per 0.1% w/w and a visble OD of ~ 10 fold less Bob
9/18/96	Thomas Baer <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	Re: Visit to NIH	Dear Tom, I am fully aware of the rush that will come with the publication, that has been a key concern of mine all summer. I agree that we (I will also run our comments by Lance) should discuss a statement of work before presenting anything to OTT. This is viewed as a collaboration so both parties needs are to be met, where reasonable. Lance wants this statement of work soon. Maybe I should right a outline of our needs and possible timelines - and you can respond by your needs and what you feel is practical. We have tried about 7 different naphthalocyanine dyes and easily solubilized them in the eva polymers we have been using (not the latest one though) by using methylene chloride as a solvent mixing into melted eva and then vacuum evaporating the solvent. We have then made films with this material. We purchased these dyes from Aldrich (expensive low volume producer). Their new catalogue has a new dye with peak at 808nm using a vandyl coordinated cation (the dye we had selected also had a vandyl) it is stated to have a high mp

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				>300°C which I interpret at high thermal stability and it apparaently high purity dye- we are trying to get some quick to see if it is as soluble in the eva's. If so I would rather have Optopower supply a 810nm LD. (Note the spectra shift in the solid eva and may also shift slightly in the melted film). - I would much rather deal with someone who supplies kgs of the stuff to the CD-ROM industry. The needed dye concentration is ~0.1% or 1mM. I am talking with the relatively small adhesive film and industrial applicator manufacturer Electroseal that I got a 100um thick eva film that I used for our latest work (I have about 1500 ft of it). It is not tacky but lies on tissue nicely - which is nearly optimum which respect to avoiding non-specific transfer. They won't tell me what isin their film so it becomes difficult to independently modify. I am also a little worried that they do not make medical tape or have a clean room - they may not be able to scale up to commercial production easily. However, they are quick to respond and been helpful to date. I am discussing with their independent compounder how to get the dye into a bench production sample (10-15 lbs) of eva for making films at Electroseal and at NIH. Everything in this project is so interconnected that basic decisions on laser wavelength and microscope configuration (inverted or upright) rely on an integrated vision of a workable clinical research system. I want to make the best decision on laser and microscope configuration right now so that OptoPower can supply the LD system and we (my NIH team) can put a test system together here in a month. This can then be used to better define the multiple brassboards you might make. regards, Bob >Dear Bob, > >Congratulations on getting the Science manuscript accepted. When will it be >published? Will there be any announcements before publications?

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				(If you >think the pressure is high now, wait till after everyone hears about your >work!!!) > >We definitely need to begin to formulate a plan so that we can make most >efficient use of our resources. I will try to call you tomorrow to begin >that discussion.... > >The next step in the CRADA is a statement of work. It would probably be >best to discuss this statement before anything goes through the OTT. Do you >feel that this is appropriate? > >I agree with your earlier comments that a good portion of the effort needs >to go into 'non-microscope' activities. Between the two groups I am certain >we can make a very good device for welding the film to the tissue. I think >that it will be challenging to optimize the film and design an easy to use >'punch out' tool that pathologists can handle. > >I have contacted Optopower and I am waiting for a response. I will contact >some other vendors that might be able to supply 840 nm diodes on short >notice. I will let you know as soon as I come up with something. > >I think other dyes should work well and I would definitely prefer a dye that >absorbed around 810 nm where we can certainly get high power diodes quickly. >Most IR dyes have comparable absorption lengths to naphthalocyanine,

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				>however, this dye has better photobleaching characteristics. I am not sure >that photobleaching is important since this is a one shot operation. I have >heard that most phthalocyanine dyes are 'rocks' i.e. that they don't >dissolve well in most common solvents. So, there may be a better choice... >Do you have a recipe for getting the dye into the film or are you going to >let the plastic people handle this? I believe that it may be possible to >dissolve the dye in a suitable solvent for the plastic and then spin coat it >onto your film. This may facilitate testing a number of dyes quickly. My >estimates are that you are going to need a dye concentration of higher than >about 1 millimolar, is that what you estimate? > >I have copies of abstracts from several patents describing the eraseable >CD's using naphthalocyanine dyes. The full patents will probably have a lot >of detail on how the disks are made, apparently they use a sandwich >technique similar to what we discussed. Would you like me to order these >patents? > >Well, obviously I still have lots of questions. I will try to call you >tomorrow. > >My best regards, > >Tom Baer > > >At 07:14 PM 9/17/96 -0400, you wrote: Dear Tom,

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				I am attaching a statement of work that I wrote up. Any comments. What did I leave out. What about longer term? >Tom Baer
9/18/96	"Thomas J. Pohida" <pohida@nih.gov>	bonner@helix.nih.gov (Robert Bonner)	interest?	Tom, There was a flurry of activity for the first 45 minutes (when the workshop sessions ended and the ccokies and coke lasted. Judy came by and was happy. However I couldn't really engage her in a discussion of the science - she is hopeless - I think she is embarrassed not to be able to engage in even a general science discussion with give and take. No such offer. Bob PS the Science paper was accepted. If you have some clear additions to the invention report we can send them to the Lance's lawyer who is putting things to getter for a Patent Office deadline on ~Sep26. We would have to do it fast. >Hey Bob, >Did you get a lot of interest at the poster session? Did anyone ask about >how to obtain such an instrument/technique? Did Dr. Vaitukaitix offer you a >section chief position? :) >Regards, >Tom >Tom Pohida >National Institutes of Health >DCRT/NSB/NRDS >12 South Drive MSC 5659 >Building 12A/Room 3063 >Bethesda, MD 20892-5659

Date	To:	From	Subject	
				>301-435-2904 voice >301-480-6241 fax
9/21/96	laserPCR	bonner@helix.nih.gov (Robert Bonner)	New disclosure on LCM recovery	Dear Lance and Mike, Paul and Seth had a good idea for implemneting a easier sample recovery. I have added a discussion of it to the end of the Laser Capture Microdissection invention report I sent you previously. Note it is the last several pages. Lance, if you could send this on to the lawyer maybe he could incorporate it into the continuation in part. Thanks. We are also developing a statement of work. Bob
9/26/96	"Thomas J. Pohida" <pohida@nih.gov>	bonner@helix.nih.gov (Robert Bonner)	LabView	Tom, Fine. Please leave a note on the computer (post-it) to remind me. Thanks. Bob >Hi Bob, >Currently, I only have LabView for a PC. As I mentioned earlier, I >purchased two up-to-date LabView full development systems, Mac and PC >versions. These new systems should be arriving shortly. Until this time, I >will be using the LabView installed on the LCM Mac OVER the network. In >order for this to be successful, it is imperative that you (I think that you >are the only person who uses the program at this time) 'quit' out of LabView >when you are done. Lately, we have just left if running continuously. The

Date	To:	From	Subject	
				>program can only be used on one machine at a time, so I will quit out at my >end when I am done. >Regards, >Tom >Tom Pohida >National Institutes of Health >DCRT/NSB/NRDS >12 South Drive MSC 5659 >Building 12A/Room 3063 >Bethesda, MD 20892-5659 >301-435-2904 voice >301-480-6241 fax
9/26/96	"Thomas J. Pohida" <pohida@nih.gov>	bonner@helix.nih.gov (Robert Bonner)	status	Tom, Attached is the statement of work I wrote for the CRADA. I think the best way to handle your involvement on the CRADA is to have Lance write a memo to Risso/Martino that a CRADA with the NCI has been established and that he expects you to be a critical component of the project. That would make you an integral part with a clear request from the NCI. How about it? Bob
10/01/96	"Thomas J. Pohida" <pohida@nih.gov>	bonner@helix.nih.gov (Robert Bonner)	status	Tom, Attached is the statement of work I wrote for the CRADA. I think the best way to handle your involvement on the CRADA is to have Lance write a memo to Risso/Martino that a CRADA with the NCI has been established and that he expects you to be a critical component of the project. That would make you an integral part with a clear request from the NCI. How about it? Bob
10/01/96	"Thomas J. Pohida"	bonner@helix.nih.gov	next week's	

Date	To:	From	Subject	
	<pohida@nih.gov>	(Robert Bonner)	meeting	Tom, We'll see when the first meeting can be arranged (I am doubtful of next week). But if Lance agrees to a fixed schedule of weekly meetings, I am reluctant to cancel the start of it. I expect that the meetings should be held on a weekly basis even if, Lance, Mike or I couldn't make it (early on they probably won't be held if only one of the 3 of us could make - or at least if both Lance and Mike couldn't make it.) But as people get to know each other I think a meeting with only Rodrigo and Christina from Lance's group could be interesting for us. I like the film on IBM selectric(?) disc or some variant. Bob >Hi Bob, > >What do you think of the 'film on disk' idea? The mechanics would all have >to be worked out, but I think that it is a potentially very clean solution. >I'd bet that Baer would respond very favorably to the idea. > >As I mentioned in today's meeting, I will be out of the office next week. > >I realize that the Optopower unit will probably be arriving early next week. >I will be writing the LabView program to control and monitor the unit. Even >though I will be out of town, I will be working on the program with my >laptop. Therefore, I don't think that my absence will impact progress to a >large extent. I understand that getting the Optopower unit functional for >testing the films, optics, etc.. is top priority.

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				> >Since the upcoming meeting with Lance's group is a first for the project, >could you please delay the meeting until the early part of the following >week (on or after 10/14)? I would really like to meet the other persons >involved. > >I'll see you in building 10 on Wednesday. > >Regards, >Tom
10/01/96	"Colby, Gary" <ColbyG@otd.nci.nih.gov> >	bonner@helix.nih.gov (Robert Bonner)	Re: Arcturus CRADA Research Plan	Gary, I attach the statement of work as a MAC MW6.0 file , and also at text below in case you can't recover my Mac MW6.0 file. Bob Statement of Work NIH (Arcturus CRADA) The NIH (NCI & BEIP[NCRR]) will continue to develop the laser capture microdissection technology and its applications. This includes developing the first laser diode microscope prototypes and special IR-absorbing films as well as optimizing methodology and its integration into molecular diagnostic testing. 0-3 months: The NIH will construct the first laser diode-based microscope system and test samples of films (single and multilayer) containing added near IR absorbing naphthalocyanine dyes. The NIH team will evaluate transfer of a variety of tissue samples with their new technology so as to define optimal parameters for transfer and range of target sizes that can be reproducibly transferred. [NIH will continue clinical research studies during this period with the existing carbon dioxide laser microscope and standard EVA polymer film - already developed]

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				1-6 months: Through consultations with Arcturus, the NIH team (BEIP) will design, develop and build 2-3 brassboards research instruments of improved design (using a fiber/optic/laser diode/controller supplied by Arcturus). NIH will investigate a variety of schemes for recovery of transfers to molecular analysis vessels. [NIH will continue clinical research studies during this period using as well the new laser diode laser microscope and new EVA polymer films]. 6-18 months: NIH will evaluate the new Arcturus research microdissection system supplied to it. NIH will operate a training program for research pathologists interested in learning the technique. The NIH will continue with the development of concepts and further refinements of the technique and its use with a variety of molecular diagnostic analysis methods. Statement of Work Arcturus (NIH CRADA) Arcturus will develop a research laser capture microdissection instrument suitable for routine research use. On a longer time scale Arcturus will develop a clinical pathology laser capture microdissection system for routine clinical use. Arcturus will provide a rugged, optimized laser diode system for early NIH brassboards systems. Arcturus will provide integrated engineering consultation to the NIH during early brassboard development. Arcturus will support all Laser Capture Microdissection instruments developed under this CRADA (in particular provide technical support and service to non-NIH sites collaborating with the NIH). Arcturus will support a post-doctoral fellow in Dr. Bonner's lab who will be working on molecular analysis techniques compatible with LCM potentially applicable to higher throughput analysis and quantitation than afforded by present techniques. Arcturus will license the NIH patents on microdissection and manufacture the systems under these licenses.

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				0-3 months: Arcturus will develop a rugged, optimized laser diode system for early NIH brassboards systems. Arcturus will provide integrated engineering consultation to the NIH during early laser diode system development. [Arcturus will investigate alternate design concepts that the NIH team cannot address and long-term clinical instrumentation requirements] 1-6 months: Arcturus will provide a rugged, optimized laser diode system for early NIH brassboards systems. Arcturus will provide integrated engineering consultation to the NIH during early brassboard development. Arcturus will investigate best commercial sources of film technology and assist NIH in developing a reliable "commercial" transfer film for use with the laser diode system. Arcturus with NIH collaboration will investigate a variety of schemes for recovery of transfers to molecular analysis vessels. 6-18 months: Arcturus will supply NIH with their new research microdissection system and support refinements in it. Arcturus will make their research microdissection system commercially available (under NIH license) and technically support such systems. Arcturus will support a post-doctoral fellow in Dr. Bonner's lab to work on molecular analysis techniques compatible with LCM and potentially applicable to higher throughput analysis and quantitation than afforded by present techniques. Arcturus will insure that there is a commercial source of film for reliable LCM transfer. The resolution limits of the technology will be determined. >Hi Dr. Bonner, > > Would you send me an electronic copy of the Research Plan you wrote? > It could simplify matters in the future. I'll be reviewing the Research >Plan and contacting Arcturus later this afternoon. > > Thanks

Date	To:	From	Subject	
				> > Gary > NCI-OTD
10/06/96	Thomas Baer <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	Re: CRADA	Dear Tom, I am sorry we played phone tag. I would have sent a email but it was complicated information and I wanted a conversation. The upshot is that we visited Olympus on Thursday. They are willing to help us (extended sales engineering) with loaner equipment, suggestions for modifications and ok to cut up their loaner equipment. Their engineering staff at Olympus America seems quite limited and we are not interested in a CRADA with them. However, they demonstrated an "automatic" pathology microscope system that AccuMed (Chicago start-up working for ~2 years) has developed. It has automated high precision mechanical stage (5um repeatability on slide), automatic slide cassette, bar code reader, and a precision tape (1mm diameter spot) deposition system which can put circle of adhesion tape at precise positions on the slides (they have a nice typewriter type ribbon system with special tape supplied by 3M), all with computer control and data archiving. The entire system is being marketed by Olympus for $17,000 (it uses a low end Olympus BX upright microscope). It seems that this system could be easily modified for LCM and a clinical system. In the NIH's interests I am going to contact them. They may be a better CRADA partner for us because they already have developed a commercial product and have an interesting engineering staff in place (they also have an reagent (?) analysis divison that Olympus doesn't know much about but may have expertise in PCR, etc.). I don't know if you could compete with such a firm (if we establish a CRADA with you, they might still beat you to market if they move aggressively). It may be that their hands are full and they would rather have a relation with you and NIH supplying their existing systems and in house prototypes (they are developing a video/digital image based system as well). I wanted to inform you and get your feedback. I will be in my pathology lab today (Sunday) from now til probably about 4PM. My number there is 301-496-1085.

Date	To:	From	Subject	
				We are trying to do a real hot, quick clinical research study with the laser system. Gary Colby has written a CRADA agreement very much along the lines of what I wrote. We are holding off sending it to you until the AccuMed discussion is clarified. You can also reach me in the evening at home 202-291-3237. Regards, Bob
10/08/96	Thomas Baer <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	Re: CRADA	Tom, I respond to pressure. Lance, Mike and I had a meeting and concurred on going ahead with Arcturus. I hope that there will be no additional delay in OTT, you should be hearing from Gary Colby with the draft document shortly. Regards, Bob Tom, I am tentatively scheduled to give a seminar at the FDA on November 18 Title: Laser Capture Microdissection and the Dawning of a New Age of Molecular Diagnosis of Disease. I may have second thoughts about such a grandiose title, but I would like to get them thinking. It might be a good opportunity to get a feel for the requirements for FDA approval for various levels of use as well as start to formulate ideas of what sort of FDA criteria would be applied to a new molecular-based pathology. Any comments?

Date	To:	From	Subject	
				Bob To: tmbaer@ix.netcom.com (THOMAS BAER) From: bonner@helix.nih.gov (Robert Bonner) Subject: Re: CRADA Tom, As expected the signing of Letter of Intent is taking a little longer than forecast. I have finished the rush prostate cancer study (or at least the first round of laser microdissections). It was really exhausting to do multiple pathology sites from 22 patients with our CO2 system. But it was a good experience to see more clearly problems requiring various technological improvements on speed and ease. The Science paper is to be published Nov 8. Yesterday the NCI had an NIH video crew in to tape a series of interviews and me doing the various steps of LCM to release to the media on November 8. My guess is that the NCI is going to make a considerable publicity effort. The NCI Deputy Director for the division including Lab of Pathology may have overstated the potential impact of LCM by saying it could be as important as PCR. Anyways the expectations are quite high, and the pressure to produce will only mount. However all this provides an exceptional opportunity if we move quickly and accurately. Olympus just brought us a BX50 microscope with "Universal Illuminator Attachment BX-FLA" (epi fluorescence attachment module) and 4 UPLAN Fl lenses (4x/0.13 inf/0.17; 10x/0.30 inf/-; 20x/0.50 inf/0.17; 40x/0.75 inf/0.17). We will try to introduce our Optopower LD fiber with collimating selfoc lens (separate purchase) attached into this system to provide an appropriate range of target sizes as objectives are changed (I expect probably 200um @ 4x to 20um @ 40x). At this moment we are trying to ascertain the best point of introduction (there are a series of lenses in the system).

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				Paul has made his own benchtop microscope with the fiber/selfoc lens incorporated for our preliminary studies of transfer and possible photochemical damage. Last June we hired Eugene Goldberg of Univ of Fla Gainesville as a suface adhesion expert consultant for 6 months. He visited in June saw our system (we were having trouble transfering at that moment but within a couple weeks had independently solved the problem). We wanted to understand better the adhesive forces we generated and how to optimize them (or reduce the adhesive force of tissue to slide to an appropriate level). Also there is the possibility of transferring living cells or cells surrounded by index matching fluids (to give a better image during the targeting) which in general is not compatible with hot melt technology (at least eva's) since they solidify at the fluid interface and do not form an adhesive bond to the tissue. In the rush of all my work this summer I have neglected to define good questions for Dr Goldberg to address. What do you think about using him further and what are some critical problems to address? Think about it and we can discuss in on the phone or when you visit. Happy Halloween. Bob
10/09/96	Seth Goldstein <sethg@box-s.nih.gov>	bonner@helix.nih.gov (Robert Bonner)	Re: Talk at 2pm Wed , 10,9 in the BEIP Conf RM, 3W54	Seth, To summarize our discussion yesterday about specific tasks on the LCM project. I would like you as soon as possible to summary the results on the finite element calculations that have been done and arrange a meeting of you, me and Phil. I want to have some ideas to guide our initial explorations of activation of dyed films in small spots. (concentration of dye; PULSE: power,duration, waveform, spot size; effect of film thickness and overlay thickness). Perhaps computational and empirical studies would best be done in parallel. I need to know the state of things to evaluate whether this approach is useful. Work with John Peterson to make certain that you have enough dyed

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				film (different OD - I expect the best to be between 0.3 and 0.9 OD for each film). What about his ordering the cyananine dye (laser dye 790)? We need these films with and without support film. Work on design concepts for adhesive wheel (with spokes) for application of small spots of adhesive film to selected regions of tissue. What sort of easy relaese mechanism can you think of? Set up micropunch system in Bldg 10. On Friday I will suggest that we do a study with the laser and Punch (trying to select a problem in which the transfer will be small and spread out on the slide so the small punch areas could be a real benefit.) This seems like a lot of work - try to give me short written reports of progress - that we can refine and then distribute around the larger group. I would like to be able to give Lance's group and Steve Bova such minireports at our Friday meetings. Bob
10/18/96	"Thomas J. Pohida" <pohida@nih.gov>	bonner@helix.nih.gov (Robert Bonner)	Labview	Tom, The system worked well yesterday. Thanks. Bob
10/29/96	laser,lab	bonner@helix.nih.gov (Robert Bonner)	New projects	John, Paul, Seth, Tom, I had sent an email to Dr Slavkin after his talk seeking to follow up on a couple of my ideas of technologies to solve his large problems in developmental biology. I had suggested that 2-photon (single if tissue not thick) photochemical activation of the promoter of green fluorescence protein (a new tool for making fluorescent cell lines) might allow the activation of the production of GFP in only a few (one) targeted cells in an embryo. Such specific targeting might allow the migration of specific cells during the critical period of neural crest formation (for example) to be precisely followed by fluorescence microscopt

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				(confocal or not depending on turbidity and background fluorescence). Glenn Nuckolls of Slavkin's lab has sent me a number of reprints. Anyone interested in being part of the preliminary discussions please contact me. John, I would particularly like you to look at the articles and think about the photochemistry of specifically turning on a promoter. In regard to his problem of imaging developing bone structures noninvasively, I suggested OCT and then contacted Joe Schmitt to see if a system could be purchased. His reply: A new resonant scanning stage produced by German company facilitates the construction of a relatively fast (10 sec for 256 x 256 image) fiber-based OCT scanner. The scanning stage was originally designed for use in OCT by Alex and I, but now it is in commercial production. I built a working prototype with this stage that is extremely easy to use and relatively inexpensive. The software is user-friendly and gives an image on the screen that can be stored or tossed out. The total cost is about 20K$ (12K$ for the stage, 3K$ for the optical/electrical components, and 5k$ for the PC and data acquisition card). Alex also has a prototype (4 channels to speed acquisition), but I don't know whether Alex and Boehringer Mannheim have made any plans yet to market an instrument. He is coming here next week--I'll ask him whether he would be interested providing a prototype instrument for use in the developmental biology lab at NIH. If not, I would be willing to help build one for you if NIH would buy the components (my labor is free for my friends!). Maybe someone in BEIP could build it with my instructions. If Slavkin and Art Levine are interested perhaps this would be a good project for Tom Pohida to build with help from Paul and me, and Amir might be involved in helping with experimental protocols and interpretation of the backscattered images? I also had a talk with Misha Ostrovsky (a member of the Russian Academy of Sciences who will be working in the NEI for the next year). He is very pleasant and interested in collaborations on ocular photochemistry. Paul and I are getting the XeCl excimer ready for

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				him to rapidly generate UV photochemistry of lens proteins and then of some knock out mice lacking alpha-crystalline. This is to explore protective mechanisms in the lens inhibit photochemical cataract formation Ostrovsky has done some pioneering recent work on lipofucsin photochemistry in the retina and its possible relation to macular degeneration of aging (an old hypothesis of mine). I am excited about this -is anyone else? Bob
11/10/96	Thomas Baer <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	Re: CRADA and visit	Dear Tom, Glad to get your message and look forward to a preliminary agenda. I would hope you and I could meet on Sunday. I think we ned to cover the ground before having meeting with a bunch of individuals, I am not the greatest manager of government workers and sometimes group discussions tend to wander. It is important that we have technical group discussions (my group) in addition to meetings with Lance, Mike, Steve Bova (Hopkins) from the medical side. I am a little worried about talking to the press (technical) and disclosing the directions we are going in. Should I be? Any suggestions how to handle it (we are NIH after all)? How should we handle the "CRADA" partner issue? I have said that we are trying to work with an outside company to issue that the technology becomes available for other research institutions within ~1year. I don't see any reason to mention Arcturus at this time - what do you think? We will give you samples of all the films we have been using when you come. Regards, Bob >Dear Bob, > >Thanks for the update. Gary Colby called me on Friday and left a

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				message >that the CRADA was signed and currently in effect. Thanks for all of your >efforts to get this signed before the craziness starts... I checked out >your article on the Science Magazine Web site. The figures look beautiful... > >I will put together an agenda for our meeting and email it to you in the >next day or so. > >I would prefer to meet with Olympus either Sunday or Monday evenings, or >before 3PM on Tuesday. I am assuming that I will spend most of the day with >your group on Monday, Nov. 18. (I will need to leave for the airport by 3 >PM on Tuesday.) > >Is it possible to get some samples of the films you are using? Both the >original CO2 film and any diode laser based films would be helpful. I have >a demo of a BX50 with motorized stages scheduled for Monday. > >I am glad to help on obtaining any optics or other items, so please don't >hesitate to ask. I have had good luck with Omega (and I know the people >there) but I can check out Chroma as well. The dichroics we are asking for >are not at all demanding. > >I will be in touch about an agenda. > >Best regards, > >Tom Baer >

Date	To:	From	Subject	
				>P.S. If possible, please give me some warning if Arcturus is going to be >involved with any of the publicity.
11/10/96	"Susan M. Reiss" <smreiss@erols.com>	bonner@helix.nih.gov (Robert Bonner)	Photonics Spectra article	Dear Ms Reiss The paper and figures are posted on Science On-Line as you probably know. I don't know how you arrange for permission to publish those photos (all authors are US govt employees), but perhaps Science will let you pull selected ones off their web site. Bob >Dear Dr. Bonner: >I know you are busy, but I would like to interview you for an article >that will be published in Photonics Spectra magazine. Our interview >would probably last about 20 minutes and can be done by phone. If you >prefer, I can email you questions and you can respond. The only >information I have currently is a short abstract of the talk you'll be >giving on Nov. 18. My deadline is next Wednesday (Nov. 13). The magazine >is also interested in photos (or electronic versions) which they'd like >to have by Tuesday. >Thanks for your time. Hope to hear from you soon. >Susan
11/11/96	Thomas Baer <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	Re: CRADA and visit	Dear Tom >Dear Bob, > >The main topics that I would like to cover during my visit are; > >1. Discussion with you on coordinating NIH and Arcturus engineering groups

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				!!We are presently configuring the BX50 to provide the LD spots to the center of the field (dynamically adjustable position independently of stage??). By changing the objective the field and spot size scale proportionally allowing finer work at high magnification. The finite element analysis of the temperature profile induced in film (mylar + EVA), underlying tissue and slide seem to pretty well describe the actual transfer sizes for a range of tested variables - We expect to do careful test of theory and empirical to try to optimize films for long term wetting (~0.5sec) of central spot targeted will minimal peak temperature and lowest possible slide temperatures (not to cook tissue onto slide) for every possible spot size 10um to 300um. I checked the image quality through our films with the BX50 and it still is better than our low end inverted microscope (CK2). Very soon we have to scale up production of chosen dye and make reproducible high quality bulk films. If the BX50 works fine with the laser diode both Mike Buck and Steve Bova would like a system pronto to begin hands-on studies by pathologists (these two are exceptional at this technique) which would include fellows etc to give a better feel for reproducibility in a variety of hands. We are working on a pressue plate to hold smaller pieces of film in the center of the microscopic field - hopefully this will make tissue contact less of an art form (other possibilities include a very mild pressure sensitive adhesive layer [Post-It idea]) the film application and removal must be made simple and reproducible without finesse. The collection of film transfer spots into a collection vial must be quick and simple - we have a number of ideas as you probably do. This interim period must develop the best and test them so that the beta test sites have a less cumbersome or complicated process and higher throughput. Image handling, roadmapping, position recording, patient and target info recording are being put into our prototype but clearly a lot of things needs to be tried and evaluated before we come with a reasonable beta test site program.

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				>2. Technical discussion with your group on the LCM instrument !!I will send attachments of some of our "invention reports which outline our thinking at this moment. It may help you get into a discussion with our group. I expect that Monday will largely be used for discussions. I don't think the FDA talk will be very formal. The announcement is as follows (1;30pm I think): Laser Capture Microdissection and the Dawning of a New Age of Molecular Diagnosis of Disease. Robert F Bonner, PhD, Head, Biomedical Optics, Biomedical Engineering & Instrumentation Program (BEIP), NIH Over the last year we (BEIP and NCI) have developed a new technique "Laser Capture Microdissection" in which specific cells are microtransferred from a pathology or cytology slide to a film from which the DNA, mRNA, and enzymes are subsequently extracted and analyzed (Science, in press). This technique marries the precision of microscopic identification and localization of tissue pathology by a trained pathologist using standard light microscopy with the power of new molecular biology techniques such as polymerase chain reaction (PCR) and reverse transcriptase followed by PCR (RT-PCR) to amplify minute quantities of DNA and RNA. With this technique, it is possible to compare changes in genomic DNA, gene expression, and enzyme activity in minute quantities of pure pathological cells with those in normal cells from the same patient. For

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				example, loss of heterozygosity (DNA allele loss) of specific tumor suppresser genes and changes in genomic DNA expression (mRNA distribution) in early precancers or in situ cancer can be determined. Other applications include identification of specific pathological organisms invading tissue (e.g., drug resistant TB) and changes in patterns of gene expression in a variety of pathologies which may be critical to accurate staging and evaluation of response to therapy in a new era of highly specific and detailed molecular diagnosis. >3. Discussion with NIH Pathology staff on defining the brass board >instrument configuration !!I think it is best if we come up with a concrete scheme for brassboard (and options) and present it to Mike, Lance and Steve. Lance can be a real loose cannon - though very nice when you don't leave him much room to blue sky it. Mike and Steve are much more practical and want the best system they can get quickly and will allow updating to include features we are testing for consideration on the beta-version. Note initial Mike and Steve could do a lot without a programmed stage and a lot of current work could easily be done with a manual x-y stage. Note: now Mike is excited about some single cell work such as selecting out sets of individual cells in mitosis within a tumor (so we do have some extremes to design for -- we might build 3 prototypes 1 for Steve 1 for Mike and 1 to be working on new features (for me). >4. Discussion with OTT on continuing the CRADA process !! I would suggest you try to make an appointment now with OTT and with Joe Harford (NCI). > >These are roughly in order of my priorities. > >I am assuming that Monday is not available due to your talk at the FDA. >What time is the talk? If there is time to schedule some of these

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				meetings >before or after your presentation I would be available. (Is it possible to >schedule #3 for some time Monday?) Certainly if you have a car you might schedule for late morning before my talk so I have a little time to prepare. > >Assuming we don't schedule anything on Monday: > >I propose that we get together on Sunday to discuss #1. !!Monday am should be spent in Beip with Paul, Seth, Tom, and John. This gives you the opportunity to follow up on Tuesday briefly. !!Tuesday we should meet with Mike, Lance and Steve. !!I would like to spend a half day on Sunday with you. That we spend >Tuesday morning with your group (starting early) on #2. See if we can >arrange Tuesday lunch with the Pathologists (#3) and OTT meeting right after >lunch (#4). I have to leave NIH by about 3 PM on Tuesday if I keep my >current flight. > >(Anything we can schedule for Sunday or Monday is fine with me...) > >Please let me know your thoughts about this schedule. > >I will email a more detailed list of topics for the four areas listed above >after I hear from you. > >I would prefer not to involve Arcturus in the early publicity if that is OK

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				>with you. FINE If you need to mention Arcturus as your CRADA partner for some >reason, please let me know. Until we can supply instruments to people the >publicity does not do anybody much good. I would prefer to keep a low >profile until that point, if we have the option. In general I have found >interacting with the technical press to be a lot of fun, I hope you enjoy it >as well. > Look forward to hearing from you. Best regards, Bob
11/11/96	Thomas Baer <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	Re: CRADA and visit	Here are two of the abbrev files as text. I think. Wed Nov 13 17:16:47 1996 To: Thomas Baer <tmbaer@ix.netcom.com> From: bonner@helix.nih.gov (Robert Bonner) Subject: Re: Upcoming visit TOM, I didn't receive the draft agenda (was it supposed to be an attachment?). I just called Reinhard Enders at Olympus and arranged for us to see him and Mack Naito (Olympus Japan) at the Neurosciences Meeting exhibit area on Sunday afternoon. I don't have a firm time yet but he will set it up when he gets to Washington on Saturday and give me a call at home. (he will get us visitor passes to the exhibit area). I am not certain about dinner with the pathologists. Mike and Lance can't make it on Sunday but Steve Bova said he was available (he lives in Baltimore). I would be happy to join you. We'll see what

Date	To:	From	Subject	
				Steve says - I think it would be useful for you to talk to Steve - but maybe we can reschedule for Monday dinner. bob >Dear Bob, > >Here is a draft agenda for the meeting with your group. Is this useful for >you? (It is for me...) Have I left out any topics? > >If this is useful to you, I can prepare a similar agenda for the meeting >with Lance et al. >Let me know if you want me to continue along this vein... > >I have made reservations for an engineer, Dave Head, to accompany me on this >trip. He will be the principal engineer involved with this project. He has >an extensive background in lasers, optics, mechanical design, materials, and >is fluent in Lab View. I have worked with him for many years... > >Best regards, > >Tom Baer
11/11/96	laser	bonner@helix.nih.gov (Robert Bonner)	Tom Baer's visit	Tom Baer will be in town to see us on Sunday Nov 17 through Tuesday 19. I suggest that he meet with me on Sunday (anyone else interested?) and then all of us on Monday. I have to give a talk to the FDA on Monday early am. He could then talk to us again on Tuesday am briefly and we could all meet with the NCI folks. He also will meet with OTT staff sometime. Paul, do you think you can have the microscope (BX50) hooked up with the LD and doing transfers by then. I think it would be good to set that as a target.

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				Seth and John, Could you come up with a plan for ordering the dye in sufficient quantity that we could send some to the compounder company that Electroseal put us in touch with? If we make 15 lbs of the dyed adhesive it should be plenty for them and for us. My guess is that the OD for 100um thick should be ~0.8 (that way we could also make thinner films for higher resolution work). We have to redo the LD transfers as the tissue DNA wasn't any good. Seth, I would like you and Phil to present your finite element modeling on Thursday if possible. Tom, it would be nice to have the updated program running by this Thursday so I can try it out and we can demonstrate it to Tom as an intro to discussing other additions. I will be at a NSF review panel Tuesday and Wednesday but be back on Thursday. I want to make the most of Tom Baer's visit and also show him how much we all can accomplish. See you on Thursday. Bob (home 202-291-3237)
11/11/96	buck, lance, gbov@welchlink.welch.jhu.edu	bonner@helix.nih.gov (Robert Bonner)	Schedule with Tom Baer	Mike, Lance, Steve, Tom Baer sent me a suggested schedule. Would you be interested in having dinner with him on Sunday? How about the rest of the schedule? Bob From Tom Baer to Bob Bonner: So far the schedule looks like this (please comment): Sunday: You and I meet for a few hours, time to be specified

Date	To:	From	Subject	
				Sunday Evening: Not scheduled (dinner with Pathologists?) Monday morning: Preliminary meeting with your group Monday early afternoon: FDA talk Monday late afternoon: Wrap up meeting with your group Monday evening: Not scheduled (dinner with your group??) Tuesday morning: Meeting with Pathologists Tuesday early afternoon: Meeting with OTT if possible (I will arrange) Tuesday ~3 PM: Departure Does this sound reasonable?
11/13/96	laserPCR	bonner@helix.nih.gov (Robert Bonner)	Tom Baer's visit	>Date: Wed, 13 Nov 1996 14:22:40 -0800 >X-Sender: tmbaer@popd.ix.netcom.com >X-Mailer: Windows Eudora Light Version 1.5.4 (16) >Mime-Version: 1.0 >To: bonner@helix.nih.gov >From: Thomas Baer <tmbaer@ix.netcom.com> >Subject: Upcoming visit > >>Date: Wed, 13 Nov 1996 09:03:15 >>To: bonner@helix.nih.gov (Robert Bonner) >>From: Thomas Baer <tmbaer@ix.netcom.com> >>Subject: Upcoming visit >> >>Dear Bob, >> >>Here is a draft agenda for the meeting with your group. Is this useful for >you? (It is for me...) Have I left out any topics? >>

Date	To:	From	Subject	
				>>If this is useful to you, I can prepare a similar agenda for the meeting >with Lance et al. >>Let me know if you want me to continue along this vein... >> >>I have made reservations for an engineer, Dave Head, to accompany me on >this trip. He will be the principal engineer involved with this project. >He has an extensive background in lasers, optics, mechanical design, >materials, and is fluent in Lab View. I have worked with him for many years... >> >>Best regards, >> >>Tom Baer > > > Draft of agenda for meeting with Bob Bonner's Group >November 13, 1996 > >I. Introductions >II. Discussion of status of CRADA >A. Signed letter of intent and implications >B. Non disclosure agreement and confidentiality >III. Review of LCM project at NIH >A. Update of progress since report in Science >1. Progress in moving toward diode laser based system >a) Film technology >b) Microscope >c) Diode source >2. Clinical studies >a) Studies performed >b) Problems with current system >IV. Current activities and future plans at NIH >A. Refinement of film design >1. Identification of suitable vendors >B. Design of film transfer holder

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				>C. Design of microscope platform >1. Manufacturer: Inverted/non inverted option >2. Fiber coupled diode >a) Wavelength/power >b) Optical output characteristics >(1) Diameter of fiber >(2) NA of fiber >3. Computer control of stages and video capture >a) Stage hardware >b) Video hardware >c) Computer platform >d) Software >D. Post list off sample processing >1. Delivery of sample to proteinase/PCR >2. Monitoring of processing for QA >E. Planned clinical studies within NIH >F. Planned clinical studies outside of NIH >V. Outline of product development plan at Arcturus >A. Technical Feasibility >B. Product Feasibility >C. Product Design and Pilot Build >VI. Open discussion on how to coordinate group activities > > > > > > > Agenda for Meeting with Bob, Mike, Lance, and Steve > > > >I. Introductions >II. Discussion of status of CRADA >A. Signed letter of intent and implications >B. Non disclosure agreement and confidentiality >III. Review of previous clinical studies performed using LCM >A. Science article data >B. Recent prostate clinical study

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				>IV. Near term plans for clinical studies at NIH >A. Types of tissue samples >B. Number and type of instruments needed >C. Volume of samples to be studied, number of transfers >D. Biochemical analysis planned >E. Plans for archiving sample images >F. Level of training of instrument operators >V. Near term plans for clinical studies outside of NIH >A. Types of tissue samples >B. Number and type of instruments needed >C. Volume of samples to be studied, number of transfers >D. Biochemical analysis planned >E. Plans for archiving sample images >F. Level of training of instrument operators >VI. Plans for in house training program >VII. Demand for instrument Beta Sites >A. Coordinating interaction with potential beta sites >VIII. General discussion >A. Projected first applications for LCM >1. Is this primarily a research application? >a) Volume of instruments >b) Number of samples/instrument >2. Foresee significant clinical use? >a) Volume of instruments >b) Number of samples/instrument >B. How much automation for initial instruments? >C. What kind of archiving would be preferred/needed for clinical or research >applications? > >
11/14/96	lance	bonner@helix.nih.gov (Robert Bonner)	Lab of Technology Development	Dear Lance, In response to our conversations yesterday and today, I want to state my enthusiasm for forming a Technology Research entity within the NCI to serve as a research and development engine to drive the development of critical technologies for molecular based diagnosis and CGAP. Such a group would require at a minimum

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				two senior investigator level positions (myself and one to be recruited - I have a potential outstanding outside candidate in mind but understand that the position would be an open recruitment). It would also include the normal postdoctoral fellow apparatus which would enable us to find young investigators in training with a background in critical technologies such as biotechnology/fluorescence techniques integrated in molecular biology. One of my principal tasks in the next couple years would be guiding the development of laser capture microdissection (through the CRADA collaboration, internal R & D, the training of beta test sites, and other external commercial licensing of as a routine) into a routine, highly specific and efficient method for acquiring pathologic and control tissues for CGAP and related studies. Aside from perfecting the LCM technique and developing its ability to target individual cells, my most critical objective is to develop the integration of sample acquisition (through LCM) with appropriately efficient, higher throughput and more precise technologies and methodologies for molecular analysis of DNA, mRNA, and enzyme activity. These include development of more efficient micro-array readers (to be accomplished through collaborations with my existing group within BEIP*) and examination of alternative technologies being developed by industry (e.g., micro chip from Affrimatrix) and academia. We would seek to most effectively utilize state-of -the-art technologies being developed elsewhere with a keen eye towards development of new technologies which in the longer term may be more practical for lower cost diagnosis of specific diseases. Clearly many of these developments will include specific fluorescent labels and novel optical detection schemes as replacements for existing radioactive assays, and the development of more quantitative analysis methods. I view the objective of such a group is solving the immediate technological hurdles to CGAP and developing a structure in which these and future critical technological needs can be met in the most creative and effective manner. I would hope that the group would clearly demonstrate to the SD's and community at large the critical role that internal technology development has in the vitality of the IRP research enterprise.

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				I believe creating such a critical mass within NCI could enable the efficient use of IRP wide resources (such as those remaining in Biomedical Engineering and Instrumentation Program, NCRR and DCRT) while creating an intellectually stimulating environment through interdisciplinary communication within which to generate the new ideas necessary for developing technologies to meet the rapidly changing demands of this effort to translate advances in CGAP to improvements in the specificity and quantitation of clinical diagnosis. *Note within BEIP a multiwavelength confocal microarray reader along the lines of the one developed for Trent and Bittner could be made for ~$150 - 200k. Bob
11/19/96	mf94f@nih.gov	bonner@helix.nih.gov (Robert Bonner)	Invention of Laser Capture Microdissection	Dear Dr. Freire, I meet you 2 weeks ago after the SD's meeting, and mention our invention of laser capture microdissection (my group in BEIP/NCRR and Lance Liotta's group in LP/NCI) and its publication in science that week (Nov 8). The Article Michael Emmert-Buck et al Laser Capture Microdissection is Volume 274, Number 5289, Issue of 8 November 1996, pp. 998-1001 and can be viewed on the www at http://www.sciencemag.org/science/content/vol274/issue5. We have signed a letter of intent to CRADA with Arcturus Engineering and they are meeting with me today to discuss our collaborative agenda. The work is patent pending with a patent relatively soon to be issued. We expect that the general patent will be licensed to many companies nonexclusively and the specific future practical implementations we develop with Arcturus under the CRADA will be licensed exclusively to them. Drs. Klausner, Rabson, and Harford have been fully informed and concur with our approach to development. We expect our method to have significant impact both in the discovery process of the multigene expression characteristic of specific pathologies and the variation within a given disease such as

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				cancer in outcome and optimal treatment. In the longer term this technique may be a part of powerful molecular diagnostic methods for routine clinical use based on highly specific molecular analysis of DNA, mRNA, and enzymatic activity. Just a note to keep you informed. Bob
11/22/96	Thomas Baer <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	FED EX PAK sent	Tom, As I mentioned to you over the phone I think a solution to the transfer of multiple small films to the same vessel (for molecular solubilization) is to delaminate the films at their contact with the plastic cap. The film will be at a highly reproducible position so this could be done "automatically" by a relatively simple mechanical device or manually by including a pull tab, say of polyester (e.g., on a multilayer such as eva/polyester/eva). Anyways this would be a variant of the basic device which would allow special collections in unique situations. Aside from easy handling of your cap idea, I think the concept of imaging the transfer while its film is mounted to the cap immediately before transferring to vessel is most important - This gives an archive specimen image and allows rejection of further processing of samples poorly targeted. Anyways the delamination may be all too easy and we may find that with strong large area bonding to the tissue or underlying slide we may have a problem with inadvertent delamination during separation of the cap from the tissue (following laser capture). It will be interesting to see how strong the bond s to the caps. I have sent the FedEX pak to you this pm should be there Monday. I included little strips of about 4 films that were not bound to the polyester. The release liner side is easily determined by trying to write on the film with an indelible marker pen (can't do it on release liner). A couple of the films are ok but most are pretty poor. A couple of runs Seth had put in oven (on release lining paper) and they became bubbly (and rough one side). I think you can iron out these defects so you made be better able to use these rejects than we

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				could.Anyways you can try. We will be making more film and could add it to the blank plugs that you send - so mainly test for feasibility and laser diode effects (you could keep all the #8 film since you have an 810nm laser diode). I am sorry there wasn't more film to send but John wasn't in and in the past we hadn't made many free eva films with dye in them. I sent spectra of the various films. Seth has made some beautiful films bonded to the polyester in the last 2 days - I think I neglected to send you a sample to see. Perhaps the formulation is Elvac 260 (for Ethylene Vinyl Acetate) and this is common nomencalature rather than TM. Bob
11/22/96	Thomas Baer <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	Dupont ElvaxTM	From the Dupont web site: There is much more there. Elvax(R) 3200 ethylene-vinyl acetate copolymer resin Extrusion Coating Resin for Flexible Packaging Description Elvax(R) 3200 is an extrudable, wax-modified, ethylene-vinyl acetate copolymer resin available in pellet form for use in conventional extrusion equipment designed to process polyethylene resins. Additives Antioxidant Applications

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				Elvax(R) 3200 is designed to provide a low-temperature peelable heat seal to itself or many other materials commonly used in flexible packaging applications. The melt properties of this resin allow it to be processed on extrusion coating equipment over a wide range of line speeds and coating thicknesses. It can also be coextrusion coated with a variety of other polymers. Elvax(R) 3200 is typically used as a lidding sealant for a variety of formed containers, in replacement of solvent applied heat seal lacquers. It will provide a good seal against HDPE film and sheet, polypropylene film, PVDC, rigid vinyl, rigid and foamed polystyrene, and nitrocellulose coatings.
12/01/96	baer	bonner@helix.nih.gov (Robert Bonner)	Thanksgiving	Tom, I hope you had a happy Thanksgiving. We will give a LCM demonstration on Thursday to Kevin Strom, the Under Secretary for Health DHHS, with the focus being prostate cancer. The positive part of this is that Varmus (NIH Director) speciifcally asked for a LCM demo [he was rather critical when Klausner had us present a LCM demonstration to Porter's House Appropriation Subcommittee in the spring - but apparently has changed his mind -- he probably has had feedback from the Science paper]. Anyways we will see how far we can get with the LD and your caps andd a pressure plate for a peak at the future on Thursday. On Tuesday, I will drive up to Polyvel to compound the 810nm dye in 28lbs of eva from Electroseal. I have been trying to reach Electroseal - because I now think we should have them put a .002" EVA film onto a .0025" mylar sheet with a strong pressure senstive adhesive (and release liner) on the opposite side. As an alternative we are looking to suppliers of pressure senstitve adhesives on mylar with release liner (we need several hundred feet roll with a width of 8-10" for Electroseal to coat - we get ~500 sq ft/5lbs EVA when coating at .002" thick). Previously we had used some samples from FLEXcon, Spencer MA ph 508-885-8200 to stick to thin bare EVA

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				films. I will try to find out on Monday what Electroseal and other manufacturers say about coating our dyed EVA on such materials or alteratively coating eva on mylaer and then adding the pressure sensitive adhesive. Perhaps your coating/plastics experts have suggestions and good leads. We expect to ship about 9 lbs of the dyed EVA to electroseal and bring the other 19 lbs home. Polyvel will try to extrude continuous 0.46 inch diameter rod to go directly in Seth's film maker with some amount of the material. I expect we would send 1/3 of the material directly to you. I expect you would like to have it in 3/16" pellets. Is that so? Paul will focus on getting the Optopower laser diode in the BX50 (epi-illumination), it turns out that Chroma did have an IR dichroic mirror is stock and will be immediately shipping us 2 Olympus cubes with them (they are only 95% reflective at 780nm and ~99% reflective at 810nm and above). We will be trying Seth's simple pressure plate to ascertain the force required and if we can lower the pulse length when we have a good pressure plate. We will use both films and your caps (which I like a lot). Aside from testing the pressure plate concept and dyed film with eva we will explore the transfer spot size minimum and the relative advantages of using epi. We will also explore the ability to irradiate through the slide and tissue (which allows us to heat at the tissue film interface most strongly and to potential use epi on the inverted scope IX50 keeping the entire top of the stage clear for mechanical gadgets, etc.). You need to keep this possibility in mind until we investigate what gives us optimal imaging and transfer. We will need to get a single mode fiber coupled LD for these tests. Can you help us? Seth would very much like to be closely interactive on the mechanical design. I want him to have this interaction - it uses his expertise and gives our NIH team intimate knowledge of the mechanical technical discussion so I can understand the trade-offs and coordinate effective feedback on design concepts from Mike, Lance and my team. It appears from what you said on Wednesday that Tom Pohida will not be designing any of the next interfaces. Please keep him in the loop so that we can discuss additional features that we want to build

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				onto the devices. Also please discuss with him the motorized stage that you want him to work on so that he can order it. It now appears to me that we will need several LD microscopes at NIH. One for the LCM core facility, one for Mike as a research device and one for my group to design special modifications (motorized stage, finest transfer spots, ... etc). In the alpha stage we will certainly need at least 2 (for Mike and for me). We (NIH) could order the scopes, camera, monitors, computers now if that enables us to have more systems as soon as possible. I think we will need to get an IX50 right away for Paul's set-up. ASAP we need to rough estimate a time line and cost for the alpha and beta test systems. I can keep that confidential (between you and me) and give a padded preliminary estimate to Lance and the OTD - the best guess estimate is largely for my own planning though I am being asked for ball park preliminary estimates - it would certainly be nice to mention to the Under Secretary and Varmus how rapidly we are going to be moving on Thursday. Tell me what you are comfortable with me saying. Finally, I am still struggling with how to use our adhesion consultant at Univ of Florida Eugene Goldberg and how to engage John Peterson. If you have any specific ideas for the questions we need to answer about the adhesive forces and how to optimize them, or anything else, please tell me. This may be particularly important if we try to bond to smaller targets or intact cells (cytology preparations) which at some point will be problematic. It may help if I find out on Tuesday waht is the eva polymer that we have been using - so that we could have discussions with the experts in the company that actually manufactures it and an extensive line orf related polymer mixtures. I noticed yesterday that 3M has a series of eva polymer films (Thermoplastic Bonding Film 560, 556,...) It is possible that the Electroseal is from 3M. regards, Bob

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12/02/96	Thomas Baer <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	Thanksgiving	Our email just came up. Dr Eugene Goldberg numbers are home 770-623-6235 ph; 700-623-6229 fax (reverse order that I told you). He generally does not communicate by email (a pity). He is in the Dept of Material Science & Engineering at Univ Florida @ Gainesville.
12/06/96	"Vaitukaitis, Judith" <JudyV@OD12A.NCRR.NIH.GOV> Cc: hank	bonner@helix.nih.gov (Robert Bonner)	Visit of Kevin Thurm to LCM lab	Dear Dr. V, Just an update on Kevin Thurm's visit to our laser microdissection lab. We had rushed to get the next generation Laser Diode microscope for LCM ready for the demonstration. It worked beautifully though we had only a day to find good parameters for its use. Lance gave a discussion of the clinical need and potential for molecular diagnosis based on LCM and then I gave a demonstration of the technology that we had developed over the last year (CO2-LCM and the new Laser Diode LCM). Mr Thurm, Dr Gottesman, and Dr Gallin (after Thurm left) each used the new laser diode microscope to transfer regions of a prostate cancer and were impressed. Mr Thurm was impressed that we have developed the technology so rapidly (13 months from my initial discussions with Lance). I think Gottesman and Gallin were impressed by the potential clinical and scientific impact (which I couldn't tell how well Mr Thurm grasped). He then went on to the NCHGR to hear Trent's discussion of array hybridization and Lippman's discussion of the medical informatics associated with the molecular diagnostic patterns of gene expressions. By the end of these presentations maybe the combined clinical impact became clear (we were the first stop and Lance and I didn't go with them to Bldg 49). Lance got word from Ruth Kirchstein that the presentation from her vantage point went very well. Bob
12/06/96	"Ramm, Louise" <LouiseR@ep.ncrr.nih.gov>	bonner@helix.nih.gov (Robert Bonner)	Re: Yesterday's Meeting	Louise, I am sorry I have been so busy that I didn't have time to draft an

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				email message to Dr V about how the demonstration to Kevin Thurm went. We had rushed to get the next generation Laser Diode microscope for LCM ready for the demonstration. It worked beautifully though we had only a day to find good parameters for its use. Lance gave a discussion of the clinical need and potential for molecular diagnosis based on LCM and then I gave a demonstration of the technology that we had developed over the last year (CO2-LCM and the new Laser Diode LCM). Mr Thurm, Dr Gottesman, and Dr Gallin (after Thurm left) each used the new laser diode microscope to transfer regions of a prostate cancer and were impressed. Mr Thurm was impressed that we have developed the technology so rapidly (13 months from my initial discussions with Lance). I think Gottesman and Gallin were impressed by the potential clinical and scientific impact (which I couldn't tell how well Mr Thurm grasped). He then went on to the NCHGR to hear Trent's discussion of array hybridization and Lippman's discussion of the medical informatics associated with the molecular diagnostic patterns of gene expressions. By the end of these presentations maybe the combined clinical impact became clear (we were the first stop and Lance and I didn't go with them to Bldg 49). Lance got word from Ruth Kirchstein that the presentation from her vantage point went very well. Bob
12/08/96	Thomas Baer <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	Recent events	We had a long conversation on Friday, but I realize that maybe I should be writing down the points we communicate for reference. 1) Gary Colby has been in a meeting on Wed through Fri (he also experienced the birth of his first child,Winston Hwang Colby, 11/22/96, 8 lb. 14 oz., 22.5 in.). He has sent me an email that he will be in touch on Monday am.

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				Topics to be covered are a) geting the CRADA established b) clarifying what is meant by "additional CRADA's" mentioned in letters from groups seeking to be beta test sites c) how we start planning for selection of beta sites and responses to other not selected as primary beta sites. d) It seems that now you think we should (as soon as CRADA is signed) refer beta sites inquiries to you as well. (I am not certain that is a great idea as there is an advantage to having one source of information which insures that everyone gets the same message). I have seen a large number of the requests for being a beta site - there are some impressive groups involved and I don't know how we are going to make the final selections. I imagine presently that in 2-months we could have alpha system here and shortly after one at Hopkins. Shortly thereafter I would like to have a preliminary meeting of potential beta site selectees (or at least a handful of them) so that they could see the alpha instruments and get a feel for what in involved. This would also be an appropriate time for getting feedback from the various groups as to what their particular needs are. I would also expect that we would provide the opportunity for at least some of the beta sites to perform pilot experiments at NIH on the alpha experiments, so that by the time we are to train the beta sites (and deliver beta systems) we can have fairly knowledgeable training sessions addressing their more important scientific protocols. APPROPRIATE USE OF THE SYSTEMS WITH OPTIMIZED METHODOLOGY IS MOST IMPORTANT FOR THEIR FIRST USE AT A BETA SITE. I also think that having such meetings might be a great opportunity for your team to visit and interact with the beta site PI's. If the NIH is the lead in contact and running the training and beta sight selection then I think it frees you from a variety of ticklish corporate/academic issues. Any comments? We will need to generate a tentative timetable and costs. There is also to be a CGAP web site which will have a technology page as well and perhaps an ability for interactive discussions. I guess we will have to be careful about what information is felt to be proprietary and not mentioned. You might generate an outline of sensitive technological issues that you are concerned about.

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				This next week I will be helping to start the development of cDNA libraries with the laser microdissection (CO2 and perhaps even LD if we can move quickly enough - in this case I will need more caps [which I expect to reuse initially]). For the laser diode system I expect to be using the inverted slide configuration with a pressure plate and your caps (below the inverted slide). We will try to determine good parameters for pressure, laser power, and pulse duration for a variety of spot sizes. We will place the ND filters in the fiber optic/ lens */ND/* filter cube beam path so that we can use the optipower system closer its relaible operating range (presently be we get good results at ~60mW setting which is not very stable). We will try to get as PC computer and controller/interface with video system as soon as possible. I will also try to get Olympus to supply a IX50 with fluorescence asap. [there are a number of application for which we would like both epifluorescence and epilaser activiation]. Please advise asap what specs on the IX50 you can see that we need to ask for in this loaner. I expect to order 2 IX50's, PC's etc with our own money unless this complicates your manufacture of alpha systems. We need to make quick definition of a LD source and its means of integration in the system. Mike was so excited by the way the BX - LD system performed (no computer or video) that it certainly is possible for us to start with a very simple system for the alpha systems (with only a LD, IX50, and a simple cap holder/positioner assembly). I would like to try your idea of a metalized eva film in the inverted geometry. We can send you 5lbs of the 810nm dye pellets. We would also like to make a variety of eva films with the materials we got from Dupont Elvax series (3 different melting points) and EMA sample try them first on the CO2 laser system. Metalized coating may affect the PCR as well as bonding strength so it might be nice to try a sample sooner rather than later.

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				I need to check on the various samples I generated at Polyvel, but I think the compounding of the dye is relatively straightforward and could be done by a variety of sources. Polyvel clearly has expertise in uniform mixing and if they don't inflate the charges they may be a reasonable supplier of services. Have you talked to the Olympus OEM person? (R Enders said that he had checked with him early on and that he had not heard from you yet). If we (i.e., you) are going to work with Olympus, I think this would be a good idea. Otherwise Enders has a split loyalty when he gets calls about LCM based on the Science paper (he has special knowledge from our NIH conversations and it would be best to co-opt his interest in independent use of that info). He is excited about the concept and thinks he has a good concept for how to position the film on the specimens and remove it simply. Researchers are calling all sorts of sources (Olympus, Adhesives Technologies, Cell Robotics) to ask if they can supply our system or an equivalent one. Getting concrete replies to potential beta site inquiries will be critical. Vogelstein sent Lance a nice FAX wanting to build a CO2 laser system immediately. We will likely demonstrate the CO2 and LD systems to him when he gives a major invited talk here next week (if he wants). He is really a big player in cancer and his enthusiasm would impress Varmus, Klausner, and Collins. I like the idea of investigating writeable CD ROM data file storage. Also Intel (though Steve Bova) is offering to give us their PC based video conferencing system. Are you interested? I think this might be a good way to run beta test site system. I also think Tom Pohida might be interested in setting such a system up. Seth would like to have a discussion about the designs he has generated and the cap handling system that your team is designing. I actually like the cap handling system as the first shot because it appears to be easier to implement quickly (I may be wrong). I think this is the most major issue currently needing to be resolved.
12/12/96	sidorova@helix.nih.gov	bonner@helix.nih.gov	Chocolate talk	Nina,

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	Cc: vap@cu.nih.gov, ralph	(Robert Bonner)		My talk next Wednesday Dec 18 (Bldg 5, Rm 405) at 1pm will be Laser Capture Microdissection and the strength of adhesion to tissue surfaces Robert F Bonner, PhD (Biomedical Optics, BEIP) Thanks, Bob
12/16/96	RJN@CU.NIH.GOV, peter	bonner@helix.nih.gov (Robert Bonner)	Medical Biophysics	** A section on Medical Biophysics, headed by Dr. Robert F Bonner (currently BEIP/NCRR) would develop new biophysical and optical technologies and methods for biomedical research and clinical applications. Current new technologies being developed include 1) Laser Capture Microdissection and molecular diagnosis, 2) activation of specific genes in targeted cells in complex multicellular tissues and embryos by photochemical activating specific promoters, 3) Optical Coherence Microscopy of tissues at depth heretofore inaccessible to optical imaging for noninvasive study of developing embryos, organ/ complex tissue culture, and transplant organs (e.g., kidney), 4) methods for quantitation of in vivo photochemistry (e.g., formation of cataracts and macular degeneration of aging) and 5) minimally invasive, 3D clinical optical imaging allowing quantitative 3D determination of the concentration of specific fluorescent macromolecular labels. The program of biophysical technology development proposed is a complex multistage endeavor illustrated by our recent efforts in the invention of laser capture microdissection (LCM) and our plans for future applications of this evolving method to a variety of critical scientific and clinical problems. In the last year Dr. Bonner has taken his original concept for LCM through a variety of feasibility tests (see Science 274:998-1001; 1996), a series of technical refinements, and finally the establishment of CRADA for commercial development. Here the focus has been multidisciplinary applied physical science with strong communication with the intramural molecular diagnostic community. As a robust routine technology base is established (including targeted single cell transfer, specialized

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				integrated LCM microscopes, and appropriate biophysical and biochemical procedures), our focus will turn to critical scientific problems such as using the technology for quantitative determination of differential gene expression in development, in natural organ function, and in pathology. This section and the laboratory as a whole has unique abilities to develop a field of quantitative multicomponent cell biology enabled by LCM and differential gene expression in complex tissues. A major, immediate effort (within a interdisciplinary group including LP/ NCI, BEIP/NCRR, DCRT, CRADA partners, and extramural multicenter institutions using LCM) will be to use LCM as a tool for clinical research into the relation between the human genome and anatomy including the differential expression of genes in normal and pathological cells selected from histopathology specimens (CGAP, GAP). In addition to understanding disease processes on the molecular level of DNA alterations (i.e., in infectious disease and cancer) and the quantitative differential expression of genes in all diseases, natural response to external stimuli, and developing systems, we will seek to develop optimized specific clinical diagnostic techniques (e.g., rapid determination of drug resistant TB and of molecular staging of precancers and cancers).
12/18/96	laser	bonner@helix.nih.gov (Robert Bonner)	Chocolate talk today at 1PM	I am giving the informal talk below to Adrian Parsegian's group on LCM in order to enter into discussion on molecular adhesive forces between the slide and tissue, the film and the tissue and the effects of glycerol and OCT (polyvinyl alcohol, peg). Come if you like. Bob My talk TODAY Wednesday Dec 18 (Bldg 5, Rm 405) at 1pm will be Laser Capture Microdissection and the strength of adhesion to tissue surfaces Robert F Bonner, PhD (Biomedical Optics, BEIP)
12/31/96	Thomas Baer	bonner@helix.nih.gov	Happy Holidays	I have worked on the draft you sent. I think we need to specifically

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	<tmbaer@ix.netcom.com>	(Robert Bonner)		address the requests of Gary Colby - as these appear to be formal requirements for a CRADA. Including the staff time committed by Arcturus, the equipment costs to be born by Arcturus, etc. Lance, we need to formally state our ideas of the alpha site and how that will be supported (by us). We should explicitly state that the beta test sites will purchase their LCM prototypes from Arcturus with the ability to trade in for eventual clinical system/upgrade. I think the outline now reflects the variety of tasks before us and probably needs fine tuning once we explicitly all of address Gary's comments.
1/02/97	Thomas Baer <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	Crada draft	This Crada document stuff is exhausting. Although it has been deleted I thought you might like to know Mike Buck's current thinking about multicenter clinical studies we would do. "Phase III - NIH Tasks Specifically, LCM will be used to procure tissue for analysis of one or more infectious agents (to be determined), and one of more genes associated with the pathogenesis of cancer (to be determined)" Of course we are thinking about drug resistant Tb and BRCA1 tumor suppressor gene loss in breast cancer as prime candidates but that may change.
1/05/97	laser (NIH INTERNAL LIST)	bonner@helix.nih.gov (Robert Bonner)	Happy New Year	Happy New Year, The CRADA paperwork was finalized and sent to the NIH CRADA committee. We now need to focus on our actual research plan and timetable. (I attach a version of a CRADA statement of work that spells out a number of our objectives - the finalize statement of work was condensed and altered fro legal reasons). I want us to write three papers quickly. A paper on the CO2 laser system in Bldg 10 (so any intermediate work can reference it and also to state our contributions), a paper on the thermal modeling and how it suggests alternatives, correct dosimetry, and thermal transients the

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				tissue is subjected to, and third a paper on single cell transfer with the epi-illumination system (we have to do it first). We have the IX50 and near to rapidly implement an epi-illumination system in it. Tom Baer is sending us 2-3 Optopower laser diodes (~400mW, 60um fiber coupled, 808nm LD) and DC power supplies for them. Tom Pohida needs to review the proposed circuit diagram maybe come up with a revised version that Tom Baer could have a printer circuit board made for. ElectroSeal is still working on making our dyed eva film (#8 dye at 809nm) - they are having problems getting the cutting of the 12in release liner to 10 inches into the stream for their cutting machines. Hopefully that will be done this week (the extrusion on the film can then be done right away). I will talk to Beamis Assoc. about future runs as soon as we see the quality of this run. I am being sent a sample of 3M #560 thermoplastic eva film (~180° F melting point) as an alternative to the ElectroSeal material. We need to explore alternatives. Tom Baer will need to focus on manufacturing methods whereby the film can be smoothly applied to the plastic caps (we have shown that the film/tissue bond can be activated with a film -tissue separation up to 15um - but not >20um when using epi-illumination through tissue and 100-200ms pulses). Transfer can be as small as 15-30um diameter presently in this mode and with a samll film /tissue separation nonspecific transfer can be eliminated. All this suggests that the film on the caps must be very smooth and uniform (i.e., a few microns thickness variation if possible) and that a mechanism to position the film "absolutely" parallel to the tissue surface and at a fixed separation of ~10um is desired. Any Ideas?? Dr Varmus's lab wanted to collaborate on a study with LCM. I would like to have a group meeting Monday am January 6 (say 11am) if everyone can make it. Please reply when you get in. Bob

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1/07/97	Thomas Baer <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	Diodes and drivers...	Tom, I received the package this am. Bob
1/07/97	Thomas Baer <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	Surface chemistry..	Dear Tom, I wanted to be more explicit about our need for some advice and direction from your polymer expert (and from Eugene Goldberg). NIH CONFIDENTIAL & PROPRIETARY 1) We are currently using SigmaCote(TM) from Sigma to form a monolayer(?) on our glass slides before applying frozen sections to minimze/optimize the adhesion of the tissue to the slide (i.e., less than the focal activated eva adhesion to the other tissue surface). What exactly is SigmaCote and how much is a monolayer? What modifications might be made to make the reduction in tissue to the slide less in a GRADED manner - i.e., optimize. 2) Could such solution coatings be applied to the eva-coated caps to lessen the nonspecific transfer between the untargeted regions of the tissue and eva film when using a pressure plate? What might we try? I project that a molecular layer coating would be dispersed in the melted eva where activated and not significantly alter the bond strength with the targeted tissue. 3) Some of the release liners literature we got from Rexam suggested some of the release liners are tin based. Float glass (the surface floated on molten tin has tin adsorbed/dissolved on it) microscope slides are being made for us. Would the "tinned" surface of the glass have less surface adhesion to tissue? Any suggestion on how to quantify that? 4) Would the metalized film added to the surface of the eva film as an absorber also act as a release agent minimizing nonspecific transfer?

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				5) Presently we have had non-contact transfer - i.e., when the 100um thick film and the tissue section are separated by up to 17um (and the film is below the tissue), the melted film still makes contact with the tissue/wets it/ and then soldifies to form a strong focal bond with it (and the film forms a surface pedestal with the tissue on it). Is this due to the volume expansion of the eva on the phase transition from solid to liquid? What is the range of fractional volume changes of eva's on melting? What role might surface tension (with the wetted tissue play in creating the pedestal in the film as it recools? NIH CONFIDENTIAL & PROPRIETARY Thanks, Bob PS.ElectroSeal has slit the mylar and Chet is trying to set up the run optimally right now - he expects to do it this afternoon or tomorrow morning.
2/01/97	sankarn@rockvax.rockefeller.edu (Neeraja Sankaran)	bonner@helix.nih.gov (Robert Bonner)	Re: verification	Dear Ms Sankaran, I thought your report was quite good and have made a quick read and a series of technical corrections which I enclude. I also attach this document as a MS Word document encoded in BinHex if you find that easier to identify the changes made (underlined). Bob Hed: Laser Dissection Aims at Cancer Cells for: NCRR Reporter, Mar/Apr 97; Critical Technology TEXT With Revisions (Bonner): Like any good detective, a cancer pathologist would infinitely prefer to catch a crime in progress rather than hunt for clues after the deed is done. A tall order, when one considers that at the earliest stages of

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				cancer, the changes occur at the genetic level, and only in very few cells. Usually a positive diagnosis must wait until numerous cells are affected, by which time a cancer is more difficult to treat. However, thanks to an innovative new technique developed by a team of researchers at NCRR and the National Cancer Institute, pathologists investigating the origins of cancer will have the equivalent of an hidden video camera at the scene of the crime Known as laser capture microdissection (LCM), the method enables scientists to target and extract specific cells (as few as two or three cells at a time) from a tissue sample in a matter of seconds and detect specific molecular changes where they occur. A tissue section covered with a transparent film is observed it under a light microscope. When the cells of interest are identified, a focused laser beam is activated. The heat of the beam melts the plastic film, causing it stick to the targeted cells, which can be lifted away, leaving the rest of the tissue section intact. The lifted cells meanwhile are subjected to the appropriate genetic or enzymatic tests, to monitor the intracellular events. "The technique gives us access to the disease, while it is still in its earliest stages, which gives us really powerful information for designing strategies for treatment" says Dr. Lance Liotta, who heads the Laboratory of Pathology at the National Cancer Institute (NCI). "Since the advent of molecular techniques like polymerase chain reaction [PCR], scientists have realized that it is possible to work with

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				individual cells and genes," says Dr. Robert Bonner, head of NCRR's Biomedical Optics, Biomedical Engineering and Instrumentation Program, and the group that developed the LCM technique. "One could think about doing experiments to see how tumor cells differ from the cells they originated-for instance how their patterns of gene expression are different or if a gene is mutated or deleted ." But for pathologists like Dr. Liotta, who worked with tissue samples from patients, such tests have had limited practical value because accessibility to pure populations of cells with the same altered genes was a problem. As he explains, "Any molecular analysis is of little value if it is conducted on the wrong cells or is contaminated by the wrong cells. It is like the computer adage 'garbage in garbage out.' In a typical biopsy specimen, we are dealing with a very mixed population of cells. So microdissection was essential if we were to get pure samples." Another reason for microdissection, he adds, was "the minute microscopic size of the cancer precursor lesions we wanted to study. Only a trained pathologist can pick these lesions out under the microscope. Once the lesions are identified there needed to be some method to capture and remove the cells of interest for study." Existing techniques for microdissection were too time-consuming and labor-intensive. For instance, one method-developed by another NCI researcher Dr. Emmert-Buck involved

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				scraping selected cells off the tissue section using very fine needles. Dr. Liotta and Dr. Emmert-Buck approached Dr. Bonner for a physical concept of how to automate microdissection. Within a year his team has put together the first prototype device consisting of a standard laboratory microscope fitted with a focused laser and using special transparent laser-activated bonding film. The NCCR team, chose to build the device around the microscope because "We wanted to maintain all of the pathologists' visual capabilities, so that they could retain complete control over their sample at all times," explains Bonner. "When Dr. Liotta first approached us, he had in mind a sort of glue that could be heat-activated by hot metal tip to stick to specific cells and thus, separate them from the surrounding tissue," recounts Dr. Bonner. "My idea was to replace this mechanical tip with a transparent film that could be placed over the tissue and activate it at specific sites using a pulse of laser." As hoped, LCM offers several advantages to its users. "Even with the most precise manual microdissection techniques, the final material for analysis would consist of clumps of hundreds of cells," Dr. Bonner says. "Using a focused laser beam, you can quickly target individual cells or very small, homogeneous groups of cells and therefore get much more specific information." In addition, the tissue sections from which the samples are obtained, remain intact, notes Dr. Liotta: "This way we are able to see and

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				record images of exactly what area was microdissected; what we got and where it came from. " This was not possible with earlier extraction methods because they either fragmented the harvested cells or destroyed the surrounding tissues. Dr. Bonner attributes the rapid development of the technique to the multidisciplinary quality of his team. "It was an intensely collaborative experience; each member offered unique skills that were essential to the development of the method," he stresses. Several factors had to be considered in the development of the technique. For instance, Dr. Bonner offers, "we needed to use a laser beam outside the normal visible range so that we would not cause any damage to the tissue." For this he enlisted the help of laser physicist Dr. Paul Smith. " In our initial experiments we used carbon-dioxide lasers because,all thermoplastic films absorb this radiation wavelength strongly and completely preventing any direct damage to the underlying tissue sample." Another aspect of the design, was developing a suitable material for the film. "We had to make a transparent film that could absorb most of the laser's energy, melt and conform to the cell's shape and cool rapidly," describes Dr. Bonner. Working with Dr. John Peterson, a specialist in adhesion chemistry, and Dr Seth Goldstein, a mechanical engineer, the team has developed an ethylene vinyl acetate (EVA) polymer films as the transfer material and special ways to make them and apply them and remove them easily to and from the tissue. Tom Pohida (DCRT) contributed his expertise toward electronic design and control of the LCM apparatus. "This technique is an example of what is best about NIH," agrees Dr. Liotta. "Each

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				component brought a key expertise and created a true synergism." Currently the team is working on improving the device. One change, says Bonner will the use of lasers with lower power, which will make LCM more compact and economical. Recently, NIH/NCRR entered a collaboration with researchers at Johns Hopkins University School of Medicine to demonstrate the applicability of the procedure. Plans are also under way to commercialize the technology, and by the end of this year, Dr. Bonner expects, "we hope to be testing LCM in ~10 major research centers across the country. Although cancer provided the main impetus for the development of LCM, researchers project a much wider application for the technique both in basic research and medical diagnostics. " Laser Capture Microdissection accomplished all our goals in a one-step "cool" means," enthuses Liotta, who has applied the technique to extract cells from prostate and esophageal cancers, as well as brain samples from patients with Alzheimer's disease. "It is a very powerful tool for studying the pathology of any disease where differential gene expression plays a role," adds Dr. Bonner. Indeed, with medical research linking more and more diseases with various genetic factors, it is difficult to envisage a situation where such a tool would not be useful.
2/06/97	gwchambers@townsend.com	bonner@helix.nih.gov (Robert Bonner)	LCM dye+laser diode EIR	Dear Mr Chambers,

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				I attach a Microsoft Word document encoded as Binhex. I hope you can handle this format. Bob Bonner
2/17/97	Thomas Baer <tmbaer@ix.netcom.com> ,head	bonner@helix.nih.gov (Robert Bonner)	Meeting on February 25th	I really don't have a feeling about whether Rick Klausner really meant the plural you when he mentioned a demonstration of LCM to his National Cancer Advisory Board. He did seem to say to Dave that he should come out again on the 25th (?). I will try to think this through and talk to Lance. It might be good as you say to get the visibility for Arcturus - however it appears to me that this visibility is at direct expense to my group {i.e., it becomes an NCI idea with a little help form BEIP and then turned over to industry). If on the other hand if I am there presenting our development work and then mention you and the CRADA - it shows what my group has done and how we are ensuring that the technolgy is transfered to the commercial sector. Can you detail how important this is to you - I think it is critically important to me. Bob >Dear Bob, > >Milton and I had a discussion about the review panel meeting on February >25th. The conclusion that we reached was that it is probably the right time >for Arcturus to get exposure at this level. If you feel it is appropriate I >would be glad to come out for this meeting. Please let me know if you think >my attendence is appropriate. > >As I mentioned on the phone I will be out of the office next week. Dave

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				>will be available to handle questions, I believe you have his number as >well: (408) 654 7931. I will be monitoring email so you can contact me that >way if you need to. > >Best regards, > >Tom Baer
2/28/97	"Vaitukaitis, Judith" <JudyV@OD12A.NCRR.NIH.GOV>	bonner@helix.nih.gov (Robert Bonner)	RE: Presentation To National Cancer Advisory Board	Dear Dr V., In response to your questions: >Thank you for the feedback. I wonder if outside news sources picked >up on your presentation since they were at the NCAB for the >mammography controversy. I don't know if any of the news media paid attention to our demo and haven't had any direct feedback from them yet. >How are you coming with higher resolution >LCM down toward just2-3 cells? I have transfered a few cells at a time (3-5 nuclei) and have targeted single nuclei with bonding but not yet reliable transfer. Paul Smith and Tom Pohida have set up one of our prototypes for us to do systematic studies in the parameter range I have used previously to target 4 um, I expect to spend the next few days trying to get reliable 4-10um transfers. >What do you think of Watt Webb's 3photon microscopy? Dr. Webb's development of his original idea of 2-photon microscopy has generated enormously excitement in the fluorescent confocal microscopy community. 3-photon appears principally a novelty unless it enables the use of new cheaper laser sources being developed in the optical communications field (i.e., Yt:Er double-

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				doped, double-clad fibers - I am going to talk to Mike Yadlowsky at Corning about this technology). In general I am concerned about the broad 2 & 3-photon absorption spectra leading to a variety of unwanted photochemistry, the high peak power densities leading to thermal transients in the cells/tissue (thermal damage), and the difficulty in achieving focused pulsed beams in thicker tissue slices (e.g., brain slices or other "organ culture" that many groups would like to study with non-invasive fluorescence microscopy). All of these (unless circumvented by modified methods based on physical understanding of the biophysics involved) may greatly limit the biologic utility of 3-D 2or 3-photon confocal microscopy. Bob
3/01/97	"Thomas J. Pohida" <pohida@nih.gov>	bonner@helix.nih.gov (Robert Bonner)	letter from Lance	Tom, Thanks for reminding me. I will draft a letter for Lance. Continue to nag me until I can confirm that Lance has sent it. I will try to use the specific items you have listed (it's good to inform Lance). Bob >Bob, >Well, the CRADA is official, and the NCI Advisory Board presentation is over. Could you please have Lance write a letter to Bill Risso and Dr. Bob Martino stating the critical role that I am playing in the development of the LCM technology. I realize that everyone is very busy, but I think that the time should be taken for this letter. I know that you are aware of this issue, and I apologize if you feel nagged by me bringing this up again. Gladly, I have been spending nearly 90% of my time on the project. I am being specifically asked to fill the electrical engineering needs of Arcturus (LD driver microcontroller design, archiving, etc..), and the Intel video conferencing effort is no small task. These are not complaints, just motivation for the letter. >

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				>Would you like to give Lance a specific list of project areas that I am responsible for completing? If yes, then the below is a list that I gave to you at an earlier time when we were reviewing my efforts and concentrations. A few of the items may not be relevant anymore, but others have been added. > >Thanks Again, >Tom > >>>>> >Electrical Engineering Tasks > >Tom Pohida > > >microscope hardware: laser diode electronic control circuitry > laser diode electronic monitor circuitry (calibration/verification) > film placement control/monitor circuitry (e.g. tape feed) > x-y table motion control/monitor circuitry > other automation sensors (e.g. temperature, humidity, position, etc..) > research optical disk technologies > >general software: application of image processing technologies > Labview programming > Optopower control/monitor for film/laser diode testing > beta system NIH data base > >computer hardware design: platform/machine selection > Labview boards > video/audio capture boards > network boards > modem (voice or ISDN) boards > motion control boards (e.g. tape feed, x-y table, etc..)

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				> memory RAM and storage > remote upgrade capability > beta system appropriate > >computer software design: Labview programming (R&D, and beta system user interface) > video/audio capture/playback software > network real-time video conferencing software > network T120 collaborative conferencing software > network record transfer > remote upgrade capability > beta system appropriate > ><<<< > > >Tom Pohida
3/02/97	"Thomas J. Pohida" <pohida@nih.gov>	bonner@helix.nih.gov (Robert Bonner)	letter from Lance	Tom, Would you prefer Lance to send a memo (hard copy) or an email to Risso and Martino? Bob
3/08/97	baer,dave,laser	bonner@helix.nih.gov (Robert Bonner)	Re: Follow-up of our conversation	NIH LCM team, Please comment on below specifications. I have included by initial comments. Bob >ARCTURUS CONFIDENTIAL AND PROPRIETARY > >Dear Bob, >

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				>Please review the following Alpha instrument definition. This is just an >intial proposal we welcome input from you and your group. > >Hope you are doing well, > >Tom Baer > > > >LCM Instrument Specifications Proposal >Thomas M. Baer / Mark A. Enright >Arcturus Engineering >5 March 1997 > >The following proposal outlines the specifications for the Arcturus >Engineering Laser Capture Microscopy (LCM) Instrument. These >specifications are proposed in order to facilitate the design of the Alpha >instruments in compliance with the CRADA signed between the NIH and >Arcturus Engineering. > >Microscope Specifications: > Microscope: Olympus IX-50 Binocular Inverted Microscope > Two 10X Eyepieces > Illumination System > X-Y Mechanical Stage Bob"s response: 4X Objective will be necessary to give larger field of view (for initial screening using ocular and maybe for transfers of most areas given the 4x additional magnification in video system - i.e. limited field of view) > 10X Objective (Standard) > 20X Objective (Standard) > 40X Objective (Optional) > Video Camera Adapter (Optional)

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				Bob's response: If video camera and monitor are optional then careful attention must be placed on targeting beam ocular visualization. That is the 808nm throughput of the ocular must be great enough to provide targeting beam visualization at ~300uW but eye safe at ~200mW > Laser/focusing optics in illumination tower > >Laser Specifications: > Laser Wavelength: 810 nm + 3 nm > Laser Power: 0 mW to 200 mW (variable, measured at the sample) > Spot Size at Sample: 60 um diameter (Standard) > 30, 60, 120, 240 um (Optional) Bob's response: I doubt 240 um spot will be used. > >Electronics Specifications: > Laser Driver: 0 to 2.5 A output, current limited > External inputs for current, modulation, and switching > Driver controls: Digi-pot output power control > Digi-pot output pulse length control > Analog variable bias set-point control > Push-button laser pulse control (mounted on microscope) > Driver input: TTL pulse trigger (negative edge) > External input for remote laser pulse switch (foot switch etc.) > Analog modulation input (1 kHz bandwidth) > Driver output: Current monitor > Indicators: Audible beep when laser fires > LED laser emission indicator Bob's response: For the shorter pulses <200msec that I use with the Arcturus system a foot switch is too slow and cumbersome. I suggest a small pushbutton box (maybe handheld) with flexible cabling to

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				power supply for LD so that pulsing can be rapid but using faster reflex time of hand and easy to find trigger (i.e., don't have to look or feel around for it). Current adjustment of dosimetry is awkward - need thumbwheel digital pots to be calibration in mW and msec - or under computer control as we have done on the epi systems. > >Video Archiving Specifications (Optional): > Camera: Panasonic GP-KR222 or equivalent > Monitor: Sony PVM-1350 or equivalent > Frame Grabber: (TBD) > Computer: PC, Pentium 100 or equivalent > Software: (Supplied by NIH) > >Mechanics Specifications: > Cap Handling: 3 position manual cap placement >a. Pickup >b. Place on slide >c. Deposit on Eppendorf vessel > Vacuum-pick cap holding mechanism > Adjustable spring loaded pressure plate Bob's response: Pressure range to be determined > Cap cassette will hold 8 caps minimum > No manual handling of caps > > Consumable Specifications: > Material: Optical grade plastic (TBD) > Clear Aperture: 4 mm minimum > Dimensions: Cap for analysis vessel > Top diameter 0.563" > Bottom diameter 0.250" > Height 0.130" > Analysis vessel: Standard 0.5 ml Eppendorf tube Bob's response: We are discovering significant differences in the physics of melting/ tissue contact/ bonding with free film as in CO2 system and rigid cap. We need to discuss this in next week's

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				conference call.
03/10/97	laser	bonner@helix.nih.gov (Robert Bonner)	Re: Follow-up of our conversation	>X-Sender: tmbaer@popd.ix.netcom.com >X-Mailer: Windows Eudora Pro Version 3.0 (32) >Date: Mon, 10 Mar 1997 16:45:31 -0800 >To: bonner@helix.nih.gov (Robert F Bonner) >From: "tmbaer@popd.ix.netcom.com" <tmbaer@ix.netcom.com> >Subject: Re: Follow-up of our conversation >Mime-Version: 1.0 > >Dear Bob, > >Here are three more items for tomorrow's phone call which is scheduled for >2 PM EST. (Please include the time zone designation, I have missed >conference calls because of misunderstandings about which time zone was >being used as the absolute reference. (Or alternatively we could all assume >GMT!)) > >Talk to you tomorrow. > >Tom > > > > >At 02:12 PM 3/10/97 -0500, you wrote: >>Tom, >> >>We will have the phone conference tomorrow with your group at 2PM. The >>following are Agenda items: >> >>1) The physics of eva bond formation in free film versus cap (the free film >>deforms on the top away from the tissue contact as the eva cools

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				and >>contracts, allowing the bond with the tissue to be formed not under >>stress). On the contrary the film as it cools remains attached to the >>rigid cap and thus the contraction of the eva on cooling places the >>tissue-eva bond under tension which frequently leads to film-tissue >>separation and non-quantitative transfer. When successful transfer occurs >>it is because the contracting eva draws material from the edges of the >>molten pedestal which make the pedestal so abrupt that transfering >>overlapping spots is compromised (compared to the free film). >> >>Any air space between the film and cap should be vented to prevent rapid >>expansion and contraction of the air space. >> >>2) The alpha prototype design definition. >> >>3) Contact with Dupont and other eva (or other polymer) formulation >>modifications. >> >>4) What about dye for commercial prototype films? The lead time may be >large. >>What are other limiting factors in scaling up production? > >5) Update on mold status and first parts delivery > >6) Feedback on spheres for self adjusting pressure plate > >7) Video archiving software plan >> >>This is a first iteration. >> >>Regards, >>Bob >> >>Robert F. Bonner, Ph.D.

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				>>Chief, Biomedical Optics >>Bldg 13 Rm 3N17 >>BEIP/NCRR/ NIH >>Bethesda, MD 20892 >>Ph: 301-435-1946 (office); 301-496-3606 (laser lab); 301-496-1085 (LCM lab) >>Fax: 301-496-6608 >> email : bonner@helix.nih.gov >> >> >> >> >
3/28/97	Thomas Baer <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)		Polyvel's head is Paul Albee 609-567-0080. It is a small organization of practical workers with Paul being the most technically sophisticated. I had talked to him about extruding a rod of larger diameter and he thought he could set up to do it.
5/03/97	"tmbaer@popd.ix.netcom.com" <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	Images of Cap transfers of Varmus slides	Dear Tom, I will ask Tom Pohida on Monday to set up the computer on the web and see about JPEG file format. I couldn't pull up the image that I sent, but it was the one I thought I was sending. It was a large tissue flap that nonspecifically transferred and did not blow off. This was the worst nonspecific transfer - it represents one of two types. This type is probably a large loose plate of tissuewhich relatively detaches en masse from the slide. This is the kind I have frequently observed with the rougher Electroseal film using the CO2 LCM. I will send you another image of the cap where the other type of nonspecific transfer is apparent. This occurs where the film is in very close contact and some pressure applied. In this case point bond strengths to the film exceed the tissue strength and a localized mist of cell fragments are transferred. This may happen with the CO2 LCM but in general the optical quality of the film is too poor to easily see it with the limited degree of inspection I usually apply to regions of the film outside the targeted transfer region (which I cut out). The third kind of unwanted tissue is not exactly nonspecific - but

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				rather poorly targeted. This occurs when the film tissue contact is very good and the laser pulse splatters the polymer onto the surrounding tissue (I think of it actually as the excess volume of expanding melted eva is forced to flow radially at the interface between the film and tissue and in these regions around the central spot is not hot enough (or fluid enough at its contact) to form as strong bonds with the tissue surface (less molecular interaction and therefore less aggregate bond force). This picks up little cell fragments separated from the surrounding tissue (failure of tissue strength as in #2 type). Finally a fourth kind of unwanted transfer occurs (I have only seen this so far with the CO2 LCM) when two cell clusters very near to one another are targeted and the intervening tissue is nonspecifically pulled up. In invasive cancers the small foci of cancer cells are interspersed with stroma containing collagen fiber. This stroma is particularly strong and if focal bonds are made with the eva film at two points the sheet in between can be pulled up (pulling up the edges of a tent). This tends to lift off all the intervening stroma (and cells it contains). I will send annotated images on Monday. Note the anotation in the upper left is a standard query option in Tom Pohida's program when the images are acquired. He is going to buy the appropriate software and hardware so that we can anotate by oral dictation (a sound bite). regards, Bob
5/29/97	Seth Goldstein <sethg@box-s.nih.gov>	bonner@helix.nih.gov (Robert Bonner)		Seth, I had missed the obvious about your membrane support idea. If we combine a fixed stop for the arm so that the membrane is stretched only a fixed amount the contact force for seating can be very light but the system robust. Last night I had beautiful transfers without any nonspecific transfer by just placing a cap on the tissue without the arm (just weight of cap). I think the problem with the cap is nearly solved.

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				Bob
7/12/97	"tmbaer@popd.ix.netcom.com" <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	BM researchers...	Dear Tom, The ATC and STO will be sponsored a presentation by Dr. Michael Powell, Director of New Technologies at Boehringer Mannheim Corporation on Tuesday April 8 at 2:30 in Wilson Hall (Building 1 top floor). The subject of the seminar was "Applications of Peptide Nucleic Acids in Molecular Diagnostics". Dr. Powell has been working with PNAs for various types of applications including PNA/DNA hybridization arrays. He also covered information about the arraying and detection system they are currently using for hybridization arrays. More people appear to want to come August 4 & 5 for a training session so I would very much like to have additional LCM instruments as well as prototype smaller caps (?), and the "video conferencing" option available for discussion. We will try to have a reasonably comprehensive LCM workbook assembled in addition to your manual. I hope you will send some of your staff. Bob
7/14/97	"tmbaer@popd.ix.netcom.com" <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	Latest LCM protocol	Dear Tom, I attach the latest version of Chetan LCM protocol. It may still be infected? Bob
7/14/97	"tmbaer@popd.ix.netcom.	bonner@helix.nih.gov	Updates	Dear Tom,

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	com" <tmbaer@ix.netcom.com> , laser Cc: arc	(Robert Bonner)		I took a few days off in the last two weeks for family trips amid a sense that things are slipping a little. I have also been pulled towards efforts to set up a new Laboratory of Integrative and Medical Biophysics in NICHD. The summer students that I have are hard working and appear to learn to use the instrument easily. This is a good sign for the use by technicians. On our end Computer issues: 1) the data accessibility is a little poor particularly for those of us not facile in windows 95. I want to have the data records transferred to a spread sheet format where data on files can easily be reviewed. Dr Linnola of the Medicine Branch of NCI came by for an introduction to the LCM device and will be a good person to work with as she presently does a lot of microdissection and comuter imaging. 2) We are also working on some standards for the stored images - in part so that archival images for publication on the CGAP website and other publications are made in one step within the LCM imaging program. 3) Tom Pohida is working on the Proshare system to see if we can have a more impressive demo for Gore and Shalala on July 24. If we can set up some sort of data sharing conferencing via the net it would be great. Along with a LCM website, these will be major structures to disseminate and build on the technology. He has the book you sent on Visual Basic 4) We need to order the automated stage ASAP as we are near the end of the fiscal year and will probably want to start working on options using it in a month or so. Please give Tom Pohida immediate advice on Pryor stages?? Beta system mechanics: 1) Your elegant idea of an easily handled adhesive cap has been very difficult to implement and taken a lot of my worry time. At this point

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				I am not certain that it will work well enough in the short term - and may require a not contact configuration to avoid non-specific transfer problems. However it seems the lighter pressure leads to more variable transfer. We have an important set of Chinese esophageal cancer slides that the first tries gave replicas (the sections were done here with our protocol).***** 2) We are expecting your new plate assmblies today. I hope they make placement easy and reliable and improve the variability in both nonspecific transfer and replicaa formation. 3) My group needs to order a good power monitor for use with the pulsed laser diode and also other short pulsed lasers such as Ho:YAG. Any suggestions? I would like to set up easily means here to examine caps and dosimetry. 4) I got a small vaccuum oven from Clinical Pathology (surplus) that we can use to try to make some prototype caps (with different eva's and dye concentrations). Could you send me your current recipe? I think the solvent coating approach will make practical the explorations of a variety of cap adhesive layers/sandwiches which may be neccessary to improve the cap approach. THERE SEEMS TO BE AN INHERENT EASE OF TRANSFER TO A FREE FILM WHICH DEFORMS AT THE TOP SURFACE AWAY FROM THE TISSUE AS IT COOLS SO AS TO PREVENT STRESS ON THE RAPIDLY FORMED BOND TO THE TISSUE. We are trying to overcome that with your rigid cap design. 5) I want to get some higher speed pictures of the wetting and release on cooling of the caps and of the free films (particularly) if I can get some very smooth films. I guess I could make some with Rainex coated glass slides - any suggestions. We have some nice videos of spot formation against glass with the interferometric fringe imaging. I would like to be able to vary the "glass surface" to access the dynamics of strong bond formation. 6) Having some test of bond formation with tissue is critical. This has been a largely ignored part of the problem- we need some quantitation.

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				7) We are experimenting with acetate slides for use with frozen sections, Coating these slides with a focally removable adhesive might be ideal. Note such a solution which is easily accommodated in frozen section making could also be used with paraffin-embedded tissue if very successful. 8) Seth has made a lot of progress with the mechanics of his small spot transfer onto a cylinder. It suffers from the same variability of replica formation as we are seeing with your caps at light pressure. MODELING: 1) Seth has completed some very nice thermal modeling of the various configurations and dosimetry. This make help us determine the important variables and standards for film manufacture as well as greater understanding of the physical process and thermal transients in tissue. I have done a series of more careful volume expansion as a function of temperature this weekend and we will integrate them into finite element models to determine the transient film expansion. PROTOCOLS & TRAINING: 1) We are developing a protocol manual and should have a good set of preliminary protocols and references by August 4. 2) We need to make certain that DNA extraction from inverted caps occurs reliably without leekage (38°C for ~8hrs) 3) Frozen sections are still a problem 4) How the machine will be used in complex core lab facility is a complex problem that will need a lot of refinement. Regards, Bob

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7/16/97	Thomas Baer <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	Support at meetings for NIH/NSF MOU	The NIH/ NSF MOU is meeting resistance from Janie Fouke head of Bioengineering Division at NSF. I wonder if you think there are any supporters for the concept at your meetings and whether you might try to elicit some outside support for the concept of Physical & Engineering Scientist scholars visiting NIH for 6months to a -1 year with NSF salary support and NIH intranmural research support.
8/15/97	tmbaer@ix.netcom.com (THOMAS BAER)	bonner@helix.nih.gov (Robert Bonner)	Re: Science Tech.Sight Article	Thanks for the 'heads up' on the science.tech article. If you are >going to use images of the Arcturus instrument, or diagrams >illustrating the film on the cap concept, then I think you should >include some attribution to Arcturus. I am very comfortable with a >statement like: This technology was developed as a part of an LCM CRADA >between NIH and Arcturus. [i.e. Arcturus does not have to be presented >as the sole contributor to the illustrated technology, however, saying >that it was a part of the CRADA implies (correctly) that we have at >least an option on exclusive rights to this embodiment, which sends the >right message to potential manufacturers...] My main concern in this >matter is that potential manufacturers not be confused or mistakenly >led into thinking that if they license the NIH patents nonexclusively >then they can copy the Arcturus design. > >Please let me know if this creates any problems. If you need any other >figures for the article (from the manual etc.) we would be glad to >supply them. > >Sounds like the next training session is shaping up nicely. Are the >dates firm? (Oct. 1 and 2) > >I will be back in the office on Monday. Will monitor email and voice >mail till then... > >Hope you are doing well,

Date	To:	From	Subject	
8/15/97	tmbaer@ix.netcom.com (THOMAS BAER)	bonner@helix.nih.gov (Robert Bonner)	Re: Science Tech.Sight Article	I do not think that it is possible to make such a direct reference to Arcturus in the figure caption. We will delete the NIH Laser Capture Microdissection text as well. We could always redraw the figure to be Seth's geometry instead. I need to know very soon (by Monday early). PS. I am really taking a flyer on the meeting announcement. Oct 1&2 is a firm possibility (the date is reserved by me for the rooms), but the use must be approved by a committee on Sept 8. I hope there is no problem. They are a beautiful set of rooms in an old convent with nice grounds for a serving food etc. I am a little concerned about the "commercial" instrument display which is probably a difficult issue - I plan to down play the Arcturus presence (you can be a helpful presence while we "provide the training" in the technique. If this turns out to be not so sensitive we can do it more like the last one (for which there was no review committee) or open announcement. Bob >Dear Bob, > >Thanks for the 'heads up' on the science.tech article. If you are >going to use images of the Arcturus instrument, or diagrams >illustrating the film on the cap concept, then I think you should >include some attribution to Arcturus. I am very comfortable with a >statement like: This technology was developed as a part of an LCM CRADA >between NIH and Arcturus. [i.e. Arcturus does not have to be presented >as the sole contributor to the illustrated technology, however, saying >that it was a part of the CRADA implies (correctly) that we have at >least an option on exclusive rights to this embodiment, which sends the >right message to potential manufacturers...] My main concern in this >matter is that potential manufacturers not be confused or mistakenly >led into thinking that if they license the NIH patents nonexclusively

Date	To:	From	Subject	
				>then they can copy the Arcturus design. > >Please let me know if this creates any problems. If you need any other >figures for the article (from the manual etc.) we would be glad to >supply them. > >Sounds like the next training session is shaping up nicely. Are the >dates firm? (Oct. 1 and 2) > >I will be back in the office on Monday. Will monitor email and voice >mail till then... > >Hope you are doing well, > >Tom
8/15/97	sgroi@helix.mgh.harvard.edu Cc: lance, mike	bonner@helix.nih.gov (Robert Bonner)	LCM Conference and Training Oct 1 &2 at NIH	Dear Colleague, </bigger>Thank you for your patience. We have been preoccupied with insuring that Laser Capture Microdissection is put in a robust, easy to use form. We are finally there. Currently the instruments and our tissue preparation and analysis methods are working well. We held a trial Laser Capture Microdissection Training Course at the NIH on August 4-5 in which we trained 16 investigators in the use of the current Arcturus LCM instrument and the methodologies we have developed at the NIH. We mixed lectures with hands-on use of 5 Arcturus LCM systems (developed through a cooperative R&D agreement with my group at NIH) and demonstrated new NIH prototypes for single-cell captures and computed control for LCM systems with simple roadmapping , data storage and digital image archiving and transmission. We believe now is the time to transfer this technology to your hands and establish an interactive network of researchers who will further develop LCM applications to molecular pathology and biomedical research.

Date	To:	From	Subject	
				We have scheduled a conference and training course : Application of Laser Capture Microdissection to the Molecular Analysis of Normal Development and Pathology October 1 & 2, 1997 (8:30am to 5PM daily) The Cloisters, National Institutes of Health, Bethesda, MD Sponsored by the National Cancer Institute and National Institute of Child Health and Human Development We will hold concurrently scientific sessions and hands-on training in separate nearby rooms in the Cloisters. From our trial course it was apparent that outside investigators could easily master, within 1-2 hours on hands-on use, laser capture microdissection of cells of interest from slides they had prepared at their home institution following our protocols. Throughout the 2 days we will have a room full of (5-8) LCM instruments available for training [attendees will reserve 1 hour training slots]. In the scientific sessions, we will cover the physics, pathology, and molecular analysis techniques underlying Application of Laser Capture Microdissection to the Molecular Analysis of Normal Development and Pathology. These sessions will include invited talks in molecular biology of disease pathology and normal development as well as presentations based on submitted abstracts. We encourage all attendees to submit a short (3/4200 word) abstract for presentation at the conference (either as 10 minute talks or posters). We solicit abstracts of research results on molecular analysis of microdissected tissue, new microdissection methods, new molecular analysis methods applicable to microsamples of 1-5000 cells, and application of molecular analysis of microdissected tissues to clinical diagnosis. The conference site necessitates limiting attendance. Please reply promptly by email of your interest in attending. We will send out a

Date	To:	From	Subject	
				formal registration form shortly. Standard protocols for tissue preparation, LCM , and molecular analysis will be available on NIH websites shortly. Sincerely, </bigger></fontfamily> </x-rich>
9/21/97	Thomas Baer <tmbaer@ix.netcom.com> Cc: pohida	bonner@helix.nih.gov (Robert Bonner)	JULY 7, 1996 Memo to Dr Klausner	e have ordered and received 3 complete computer systems that are not currently in use and are awaiting the next delivered systems (we have, I think, ordered 2 more backups which have not yet been received). Tom Pohida has downloaded an updated Metoer driver but I am not certain if he was able to test out your software with it on Friday to see if that was the only problem. He will certainly have done so by Monday. I really liked Tieng Yap (PhD) the new DCRT database specialist and think he will be a great addition to the team. Regards, Bob >Dear Bob and Tom, > >Thinking ahead to the training session... > >Do you guys have 5 computer systems to cover all 5 training stations >(three you currently have and two that we are going to ship to you)? >Please let me know ASAP if you do not... . > >Since image archiving was not part of the last purchase order, we are not >planning to ship computers, video boards, or Lab PC cards with the next two

Date	To:	From	Subject	
				>units. > >Russ is coming by on Tuesday to show us the latest version of the VB >program. It is high priority for me to get you guys on the air with this >latest version of the software. I will make this happen next week. I hope >the 4.0/4.03 Meteor driver problem has been solved, please let me know if >this is still an issue. Thanks for the input on the data base. This is >exactly the right time to begin these discussions. After we complete the >basic instrument control and video data collection and review modules we >will want to start on the data base module. I am very comfortable with >taking suggestions and input from NIH 'informatics' specialists in this area. > >Please let me know about the computers. > >Best regards, > >Tom Baer
11/10/97	tom	bonner@helix.nih.gov (Robert Bonner)	LCM website	Tom, Could you write a paragraph and outline the current and soon to be developed computerized system in the LCM core lab for the Website. Maybe you could just outline all the features we have and the hardware as well as those upgrades we are envisioning (such as a Bx50 imaging station elsewhwere, a central server, automatic LCM, etc)
11/13/97	"tmbaer@popd.ix.netcom.com" <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)		Dear Tom, >Dear Bob,

Date	To:	From	Subject	
				> >I have no problem with listing representative LCM institutions on your Web >site. I would ask that we try to be neither all inclusive nor exclude >anybody that might want to be listed. My goal is simply to avoid giving >our competitors an accurate list of all our customers. My desire to have a network of geographically distributed site that interested researchers could visit (selecting according to convenience). >(Feel free to assure Klausner and any interested members of congress that >we are fully capable of supplying LCM technology to anybody in the world >who wants to place an order. If we start to have delivery problems you >will be the first to know, I'm sure.) I was trying to give Klausner concrete numbers that the technology was rapidly being disseminated around the country. This was Klausner's and Liu's fundamental priority for the CRADA (that NIH wasn't holding onto or limiting the dissemination of a critical research technology - of course we have done the opposite spent a considerable effort to extemely rapidly make a robust technology broadly available). >I thought the Proshare session went very well today. We can clearly >accomplish much more in this format than we can with a simple conference >call. Steve often offers a very valuable perspective. I will let you make >the call as to whether we should have him as part of these weekly meetings. > It is not clear to me that what we are discussing is relevant to his >interests...

Date	To:	From	Subject	
				I want a renewed spark between our two groups to make sure we are sharing critical problems and understandings. I do not feel comfortable talking on many of these technical innovation points with Steve [there are even some points that I do not want Lance involved in at this point]. Tom Pohida was talked to Steve and told him at the last minute about the conference which left me lurching to shift the discussion towards points of his direct interest and that I felt comfortable discussing in his presence. >I have spoken with John Fahner-Vihtelic and he is finishing up details >surrounding sending Arcturus the relevant patent application. I understand >that you are coordinating who will be receiving our disclosures and will >let me know where to send them. I talked to Dr Gordon Guroff, who is the Technology Development Officer of NICHD along with his research work. gordong@helix.nih.gov name: Gordon Guroff alias: gg17e phone: 496-4751 address: 49/5a64 ICD: nichd fax: 402-2079 title: Chief, Section on Growth Factors Bldg 49, Rm 5A64 NIH Bethesda, MD 20892 > >I now have time to start on our more formal CRADA summary. I assume this >is still needed??? I think it would be a good idea to write a formal document as required by the CRADA and assess what has been accomplished of

Date	To:	From	Subject	
				the CRADA statement of work at this time (~9months). >I will probably be out in Washington for the Cell Biology meeting around >Dec. 11. Is that a convenient time for me to stop by?? >I would like to spend some time with you and your group, if you are available. It would be great. We will block out time to meet your schedule. >Has Tom received his stages yet? No. "Opelco called to give me an update on the microscope stages. The ship date has been delayed 3-4 weeks. The new ship date is ~11/24. I'll try to obtain the interface manual now. If I can get my hands on the manual, I may be able to start the development without the hardware." Tom Pohida >Hope you are doing well, Fine. Though I really need to hire a couple of postdocs. > >Tom Baer Regards, Bob
11/19/97	"tmbaer@popd.ix.netcom.com" <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	Bioengineering Symposium?	Tom, I have been involved with this process and we will have a LCM displayed as a "poster" showing what Intramural NIH "Bioengineering" can accomplish. Bob

Date	To:	From	Subject	
				>Dear Bob, > >Is this something that we (i.e. the CRADA team) should be involved in? >Sounds somewhat relevant to your program and they appear to be looking for >suggestions for topics... Let me know... > >Tom > > > >>Return-Path: <ebaldwin@osa.org> >>From: "Baldwin, Elizabeth" <ebaldwin@osa.org> >>To: "'Baer, Thomas'" <tmbaer@ix.netcom.com> >>Subject: Bioengineering Symposium? >>Date: Tue, 18 Nov 1997 17:40:15 -0500 >> >>Are you involved with, or will you be attending, the "NIH Bioengineering >>Symposium" Feb. 27-28, 1998 in Washington, DC? Info can be found at: >> >>http://www.nih.gov/grants/becon/symposium.htm >> >>I notice they're planing at least one panel on "Imaging." Liz >> >> >>Elizabeth Baldwin >>Public Policy Manager >>Optical Society of America >>2010 Massachusetts Ave. NW >>Washington, DC 20036-1023 >>202-416-1418 >>FAX 202-416-6134 >>ebaldw@osa.org >>http://www.osa.org/ >>

Date	To:	From	Subject	
				>>Now accepting applications for the 1997-98 OSA/MRS Congressional Science >>& Engineering Fellowship. Work for a year on Capitol Hill for a >>congressional committee or member of Congress. Catch Potomac fever! >>http://www.osa.org/aboutosa/policy/news/flyer98-99.htm
1/03/98	tom	bonner@helix.nih.gov (Robert Bonner)	ISDN Line in laser lab	>X-Sender: vnorman@POserver-v.nih.gov >Mime-Version: 1.0 >Date: Tue, 30 Dec 1997 14:21:46 -0500 >To: bonner@helix.nih.gov >From: vnorman@box-v.nih.gov (Vivian Norman) >Subject: ISDN Line in laser lab >Hi Bob, > >Greg Dolan with Bell Atlantic, left me a voice mail message that the ISDN >line was dropped in the laser lab and working. He installed it on the >shelf in between Sony and Dell monitors and microscope on the right. The >telephone number and circuit number are on the box. If you have any >problems, call 611, and ask for Greg. > >Happy New Year, > >Vivian > >Vivian E. Norman >Laboratory of Pathology, DCS >National Cancer Institute >496-2446
1/11/98	"tmbaer@popd.ix.netcom.com" <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	CRADA Report	<x-rich>Dear Tom, I am sorry it has taken so long for me to reply to your draft of an

Date	To:	From	Subject	
				interim report. I have rewritten the base of the report from my point of view. Unfortunately the tables lost their format and though I want to make a few changes in them I do not have a good format to work with. Please tell me frankly what you think. I would suggest we put a final version together and jointly sign it and submit to NIH. Perhaps most important is to come up with a detailed list of objectives for the next year with our new vision of what needs to be done. We had an excellent LCM Core Lab meeting on Friday morning, Lance is really helping move on a variety of preliminary clinical studies (quantitative immunochemistry of LCM cancer specimens, our colon cancer study, Mike's 3D study of molecular changes in prostate and breast cancer). He agrees with the idea that Alex Lash and I discussed to do a test study of drug resistant TB (low titier in tissue) using LCM. Mohinder Kang seems quite good and excited and I have brought together the necessary people in the core lab (as per my email to Sue Ann this morning - I am still having a little trouble getting the appropriate tissues we will see how helpful Steve Bova and Hopkins will be). Carlos Suarez will be starting in a week or so and I think with his help we could move on the fetal cell targeting from maternal blood. You wrote on Dec 15: Over the next year Arcturus is planning to release a second generation PixCell unit that will incorporate some of the improvements that both

Date	To:	From	Subject	
				our groups have been working on. These include: · Reduced beam size of roughly 5 microns, · improved electronics (including switching to an RS232 interface), · Simpler and improved visualization procedures, · Better reagent vessel capping mechanics, · Improved vacuum chuck and translation stage that allows use of a 40X objective, · Epi-fluorescence option, · Improvements to the imaging archiving and instrument control software. THESE SOUND GOOD Simultaneously we will be working on an automated LCM instruments that will allow fluorescence scanning of the tissue samples, multiple slide handling mechanics, and improved film carrier mechanics to improve sample throughput (including looking at the convex and other film carrier geometries). Our

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				goals are to have the next generation PixCell ready by summer and Beta versions of the automated system ready by the end of next year. Both of these goals are aggressive. Continued support and collaboration from NIH CRADA team members will be important to meet this schedule. WHAT SPECIFICALLY DO YOU NEED MEMEBRS OF MY TEAM TO DO? IF YOU CAN HELP ME DEFINE TASKS FOR SETH, PAUL AND TOM POHIDA [AND ADDITIONAL PEOPLE I HIRE] IT WOULD BE OF CONSIDERABLE HELP. I would also very much like our two groups to collaborate on specific assays that would benefit from LCM. These include PAP smear analysis, blood smear analysis (such as fetal blood cells in enriched peripheral maternal blood samples), and collaboration with outside organizations on specific applications such as colorectal cancer. I hope that your postdoc will to contribute to these efforts. ME TOO. I ALSO HAVE KANG AND SUAREZ IMMEDIATELY (SEE ABOVE)

Date	To:	From	Subject	
				In general I think we have orchestrated a remarkably productive collaboration, and I feel that this is no small feat!!!! However, one area we need to improve is the recording and reporting of inventions and important intellectual property. So far we have not run in to major problems, however I think it is inevitable that problems will arise. We will need to agree upon a standard method for tracking inventions so that negotiations between the two parties can proceed with well understood ground rules. I AGREE IN PRINCIPLE BUT HOW SHOULD WE PROCEED. SPECIFICALLY WHAT SHOULD WE DO ABOUT THE TAPE IDEAS THAT I TOLD YOU ABOUT. I THINK THAT IF WE CAN VERIFY THAT SUCH FILMS ARE MANUFACTURABLE (SETH HAD A SIMILAR BUT DIFFERENT MODIFICATION THAT MIGHT BE EASIER TO MANUFACTURE), THEN MAKING SOME PROTOTYPE AND LOOKING AT AN APPRORIATE TAPE HANDLING SCHEME WOULD BE A GREAT LONG TERM PROJECT FOR SETH TO WORK ON UNDER THE CRADA). DID THE NEW POSITION OF FAHNER VIHTELIC ON LICENSING MAKE THIS INTELLECTUAL PROPERTY SITUATION EASIER? DOES IT SUGGEST A DIFFERENT LESS COMPETITIVE FORMAT? Tom,

Date	To:	From	Subject	
				I am uncertain whether anyone will license LCM given the lead Arcturus has. I think peole will probably try to come up with an alternative technology. Consequently our greatest long term protection is to jointly move very quickly on integration of LCM improvements with a variety of analysis techniques and clinical applications so that the successes make a hard target for another technology. Of course, we must be careful not to choose things that can easily be done by an alternative method that does not need LCM level precision of separation. Regards, Bob My rewrite of the CRADA Report follows: <fontfamily><param>Times</param><bigger><bigger>NIH/Arcturus Engineering Cooperative Research and Development Agreement Interim Report January 10, 1997 The CRADA between NIH and Arcturus was formally agreed to on March 5, 1997 and is scheduled to run for 2 years. At the time of agreement, Arcturus and NIH had been jointly discussing for several months the Laser Capture Microdissection technology conceptualized and developed at NIH in order to develop a research plan that would provide a commercial version of this potentially critical new technology to the worldwide research community in as robust and easy to use form and as rapidly as possible. In these preliminary

Date	To:	From	Subject	
				discussions, Arcturus had proposed a particular design scheme in which the polymer film was attached to the flat undersurface of a vial cap to facilitate the integration of the laser capture step with the molecular extraction steps needed for subsequent molecular analysis. A number of alternative integrated design schemes had been proposed by the NIH team including special tape handling mechanisms and programmable stages with automatic handling of the captured material. The initial research plan developed for this CRADA had an initial focus on implementing the NIH design schemes for a laser diode microscope and IR-dye containing EVA polymer transfer surface and the Arcturus cap handling mechanism. Further projected tasks included explorations of alternative designs to insure both the development of a robust first commercial LCM prototype should the cap LCM scheme prove unreliable (for instance exhibit too much nonspecific contamination due to its large contact area) and to provide a broader base of design with which to develop LCM refinements needed for specific tasks such as single cell transfer and routine clinical applications of LCM. The first year of the CRADA has been enormously successful due to the integration of efforts of the R&D team within the NIH and the commercial product development team at Arcturus Engineering, Inc. Through a number of design modifications and evaluations, a robust commercial prototype LCM microscope was developed under the CRADA by June 1997 using the Arcturus CapsureTM design concept. Commercial development of the Arcturus CapsureTM design required transfer of the NIH technology and supplies of ELVAX 410 EVA polymer films containing naphthalocyanine and an extensive Arcturus product development of the methodology of uniformly coating this polymer onto the precision transparent plastic caps. NIH developed computer control and data archiving software proved to be highly effective and desirable in use in the training of over 100 investigators from around the world that occurred in two NIH sponsored LCM conferences held at NIH on August 4-5 and October 1-2. These demonstrations led to a commitment by Arcturus to develop and offer a supportable commercial software product modeled after the NIH program. Arcturus sold the first commercial LCM microscope in June 1997 and in October 1997 announced

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				normal commercial availability of LCM microscopes with 90 day delivery. The NIH R&D team has continued to develop alternative designs which demonstrated the potential for single cell transfer using epi-irradiation and concentration of multiple individually targeted cells onto a conical surface. The NIH bioengineering group has developed a thermal model of the laser activation process and greatly expanded the physical understanding of the LCM process so as to form the basis of knowledge necessary to design new approaches to specific scientific and clinical applications. The NIH scientific team has established a LCM Core Lab for outside investigators and used it to develop a variety of robust protocols for LCM and subsequent molecular analysis which have been shared with the scientific community through publication on a LCM website supported by NIH. The NIH team has demonstrated a variety of research applications of LCM, held open LCM conferences at NIH, and published a number of articles on LCM. Currently the NIH Core Lab is initiating a number of studies to characterize the potential for LCM in clinical diagnostic applications. Arcturus Engineering, Inc. has demonstrated its ability to develop, manufacture, and provide field support for robust commercial LCM microscopes and precision disposable transfer film on vial caps. They have nonexclusively licensed the core NIH LCM patents, paid licensing fees and begun regular payments of royalties to NIH. Arcturus has given the NIH three prototype LCM microscopes (placed respectively in the NIH LCM Core Lab, the NIH LCM R&D Lab, and with the Johns Hopkins MI Pathology Collaborating Unit) to facilitate development of the technology and its applications. The tables below contain a summary of the CRADA goals and their current status. The primary goals of the first portion of the CRADA included:

Date	To:	From	Subject	
				Developing a commercial version of the Laser Capture Microdissection (LCM) technology, Providing a commercial source of LCM film, Developing a reliable set of tissue sample preparation protocols. All of these goals have been achieved. Secondary goals included: Research focusing on developing single cell transfer capability, Mathematical modeling of the LCM process, Constructing prototypes of automated versions of LCM instruments. Substantial progress has been made on these goals during this time period as well. Beyond the stated goals in the CRADA, NIH held two LCM training sessions that were attended by 130 scientists from around the world. At these sessions the attendees received instructions on the basic theory and practice behind LCM and were able to spend time microdissecting their own samples using Arcturus commercial instruments and NIH prototype systems. Most of the groups contributed to the conference by delivering short papers discussing potential applications for LCM. Feedback from the attendees was very positive. Many of the attendees have already acquired LCM instruments and the technology is now in routine use at over a dozen institutions around the country. In summary, the primary objectives of the CRADA were to refine the LCM technology and develop a robust commercially available

Date	To:	From	Subject	
				version for use in the broad research community in as short a time as possible. This was accomplished within 6 months of the final signing of the CRADA through a close collaboration in addition to effective technology transfer. The goals of the detailed research plan have largely been accomplished by a flexible reassigning of tasks as needed and by a concentration on the primary objectives. We have moved close to our secondary technical goals of precise single cell transfer with improved targeting specificity which continues to be a combined CRADA focus. We continue to seek to better understand the underlying physical processes and the requirements for reliable molecular analysis of DNA, mRNA and proteins with LCM from a large variety of specimens. We now see a set of new foci for the CRADA effort centered around optimizing the LCM technology for a variety of new applications such as single cell transfer and routine clinical applications of LCM and quantitative molecular analysis. Many of these refinements require integration of LCM with specific imaging and molecular analysis techniques to insure accuracy, quantitation, high throughput and economy of the combined procedures. </bigger></bigger></fontfamily> >********** >NIH/Arcturus Engineering >Cooperative Research and Development Agreement >Interim Report >December 15, 1997 > > >The CRADA between NIH and Arcturus started in March, 1997 and

Date	To:	From	Subject	
				is scheduled >to run for 2 years. The tables below contain a summary of the CRADA goals >and their current status. The primary goals of the first portion of the >CRADA included: > >· Developing a commercial version of the Laser Capture Microdissection >(LCM) technology, >· Providing a commercial source of LCM film, >· Developing a reliable set of tissue sample preparation protocols. > >All of these goals have been achieved. Secondary goals included: > >· Research focussing on developing single cell transfer capability, >· Mathematical modeling of the LCM process, >· Constructing prototypes of automated versions of LCM instruments. > >Substantial progress has been made on these goals during this time period

Date	To:	From	Subject	
				>as well. > >Beyond the stated goals in the CRADA, NIH and Arcturus collaborated on >organizing two LCM training sessions that were attended by 150 scientists >from around the world. At these sessions the attendees received >instructions on the basic theory and practice behind LCM and were able to >spend time microdissecting their own samples using Arcturus commercial >instruments and NIH prototype systems. Most of the groups contributed to >the conference by delivering short papers discussing potential applications >for LCM. Feedback from the attendees was very positive. Many of the >attendees have already acquired LCM instruments and the technology is now >in routine use at over a dozen institutions around the country. > >During the next stage of the CRADA Arcturus and NIH will follow the basic >outline described in Phase II and III of the original document. As a part

Date	To:	From	Subject	
				>of these stated goals NIH will: > >· Continue to investigate improvements to LCM instrument design including >adding automated stages, >· Add fluorescence detection of labeled cells, >· Study different approaches for optimized single cell extraction. > >Working with NIH, Arcturus will: > >· Design an automated LCM system suitable for high volume clinical research >studies, >· Improve the resolution of the current LCM system to facilitate single >cell transfers, >· Continue to provide LCM instruments and film to research institutions >around the world. > >Also as a part of the CRADA NIH and Arcturus will:

Date	To:	From	Subject	
				>
				>· Collaborate on optimizing LCM for specific tests, such as single cell
				>extraction in blood or tissue smear samples and live cell extraction from
				>tissue cultures,
				>· Develop prototype fluidic devices to facilitate DNA/RNA analysis of small
				>tissue volumes,
				>· Collaborate with outside companies and research institutions to develop
				>specific DNA/RNA tests employing LCM.
				>
				>Stage I CRADA
				>
				>NIH Goals
				>
				> Goals: Status:
				>Develop Epi-illumination system Completed: Epi systems designed and tested
				>Evaluate requirements for tissue/film proximity Completed: Tests performed
				>and numerical model developed

Date	To:	From	Subject	
				>Optimize dye solvation in EVA polymers Completed: EVA/dye compounding >protocols studied and procedures optimized >Develop finite element model of thermal transients In progress: Numerical >model developed and is undergoing testing >Evaluate noncontact LCM methods In progress: Initial experiments performed >and model developed >Specify initial sample prep protocols Completed: Developed and published >protocols for standard tissue histopathology microsections >Develop and evaluate LCM platform with automated stages and video archiving >In progress: Video archiving program developed, automated stage evaluation >in progress > >Arcturus Goals > >Goals: Status: >Develop an LCM instrument based on an inverted microscope with integrated

Date	To:	From	Subject	
				>fiber-coupled laser-diode Completed: System designed in collaboration with >NIH, design finalized and released to production >Incorporate dyed films into caps of reagent vessels Completed: >Fabrication, quality control, and packaging optimized, currently in production >Develop pressure plate and positioning mechanism for handling caps and >sample Completed: Design optimized, tested on a variety of tissue samples >and incorporated into LCM system >Research alternative optical designs for higher resolution In progress: >Cylindrical geometry breadboard system constructed at NIH, 5 um diameters >demonstrated. Smaller beam size incorporated into PixCell system, >undergoing testing. >Identify vendors for manufacturing film Completed: Two film vendors have >been identified and sample films are being tested >Develop beta version of image archive software Completed: NIH team >developed LabView version for Alpha units. Arcturus team developed Visual

Date	To:	From	Subject	
				>Basic version, incorporated into production units. >Specify initial sample prep protocols Completed: Published NIH protocols >for standard tissue histopathology microsections > > > > >Stage II CRADA Goals > >NIH Goals > >Goals: Status: >Develop several LCM brassboard instruments Completed: Two Epi systems >designed and tested >Investigate resolution limits of LCM approach In progress: Epi system has >achieved spot sizes of roughly 5 microns, single cell transfers demonstrated. >Investigate methods for transfer of samples to molecular analysis vessels

Date	To:	From	Subject	
				>In progress: NIH has evaluated cap/film approach and is developing >cylindrical geometry concept >Assess quantitative recovery of DNA, mRNA, and enzymatic activity in LCM >samples In progress: Preliminary data on DNA and mRNA content in a number >of model systems has been collected. Studies are currently in progress >Evaluate the utility of incorporating automated stages and video arching In >progress: Video archiving system has been developed, automated stages have >been ordered and will be integrated into a prototype for evaluation >Coordinate development of standards and standard methods for LCM In >progress: Standard slide prep and molecular analysis protocols have been >developed and published, approaches to LCM standard samples are being >discussed, development of CLIA protocols is planned > >Arcturus Goals >

Date	To:	From	Subject	
				>
				>Goals: Status:
				>Develop LCM research platform based on alpha site feedback Completed:
				>Commercial LCM units currently for sale
				>Develop commercial sources of LCM film technology, evaluate quality and
				>reproducibility of films Completed: Two new sources of high quality LCM
				>films have been identified and films have been tested
				>Evaluate schemes for transfer of cellular material into reagent vials In
				>progress: Flat cap geometry studied and proven to be effective method,
				>other geometries (cylindrical, microtiter plate, free film, noncontact)
				>currently being studied
				>Design of pilot production facility for cap volumes of several hundred to
				>several thousand per day Completed: Pilot production facility currently
				>manufacturing caps with a capacity of over 500 units/day
				>Investigate long term clinical instrumentation requirements In process:

Date	To:	From	Subject	
				>Collecting user input from NIH and other facilities >Provide engineering consultation to NIH In progress: Arcturus has assisted >in optical design of small beam systems and improved visualization schemes > </x-rich>
1/31/98	Seth Goldstein <sethg@box-s.nih.gov>	bonner@helix.nih.gov (Robert Bonner)	Re: Transfer data	Seth, The OD measured was ~0.40 for the actual 45µm thick polymer on cap. RE: our discussion of the integral of the temperature elevation along the z-axis. Kaleidagraph does have an integral macro (under macros) which will integrate one column (y) with respect to another (x) and put the output in another column (this is Simpson's rule integration not requiring a fit. Similarly you can evaluate the derivative of a curve or smoothed curve. Note for transfer-NOT REEPLICA- we need a certain minimal volume expansion out to a given radius r(transfer) where the vol= 1/4r^2 * (vT+s) where T is the tissue thickness 5µm, v is the void volume fraction (est. = 0.66), and s is the separation of the film and tissue. I would assume that the tissue offered a polymer accessable space of 66% and that unless this is filled the bond will not be strong enough to actually transfer tissue - or at least quantitatively. Frequently we get release of tissue from the outer rim of the "wetted region - most likely because of only partial penetration of the polymer into the tissue. Thus it will be necessary to model that the integral expansion exceeds the accessible volume between the film surface and the glass [1/4r^2 * (vT+s)] up to a certain radius which corresponds to the radius of the transfer. Bob

Date	To:	From	Subject	
2/07/98	Thomas Baer <tmbaer@ix.netcom.com>	bonner@helix.nih.gov (Robert Bonner)	Re: Thanksgiving	Dear Tom, I will see you today. 3PM is a good time for me. I hope we can review, the CRADA report summary, and define some specific new tasks fro the next year, and at least suggest how to handle the intellectual property issues. I talked to Lance about the Japanese meeting and he thinks it would be fine for you to say something about the commercial technology. He says this is the top group of researchers from Japan so it will be an important forum. H e has not scheduled any evening dinners etc as he doesn't have an easy means to pay for them. It seems a little complicated to have Arcturus officially sponsor a dinner - however given the void you might be able to invite some of the Japanese participants to some "social setting". I will be out of town all that weekend (starting Friday evening. Bob >Dear Bob, > >I assume we are still going to meet mid afternoon on Saturday (3 to 3:30 >PM) and also get together on Sunday morning. Let me know if there is a >change in plans... > >Rachel said she was sending you a copy of the manual. She was adament that >she would not send a work in progress... Let us know if you have. any >suggestions. We also have a first draft of the software manual that you >might want to look at. > >I am looking forward to seeing you on Saturday. >

Date	To:	From	Subject	
				>Best regards, > >Tom

SF 1301802 v2

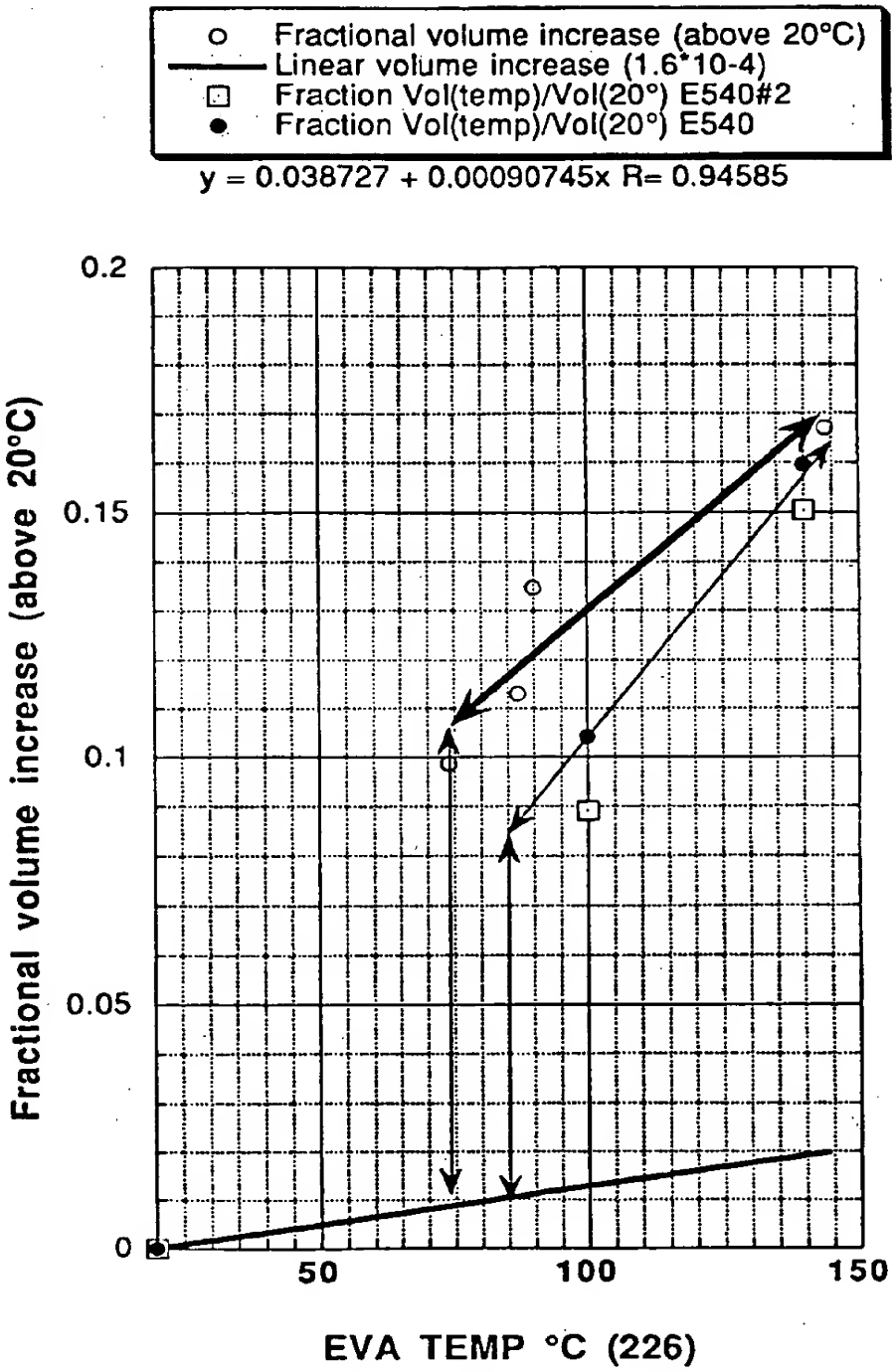
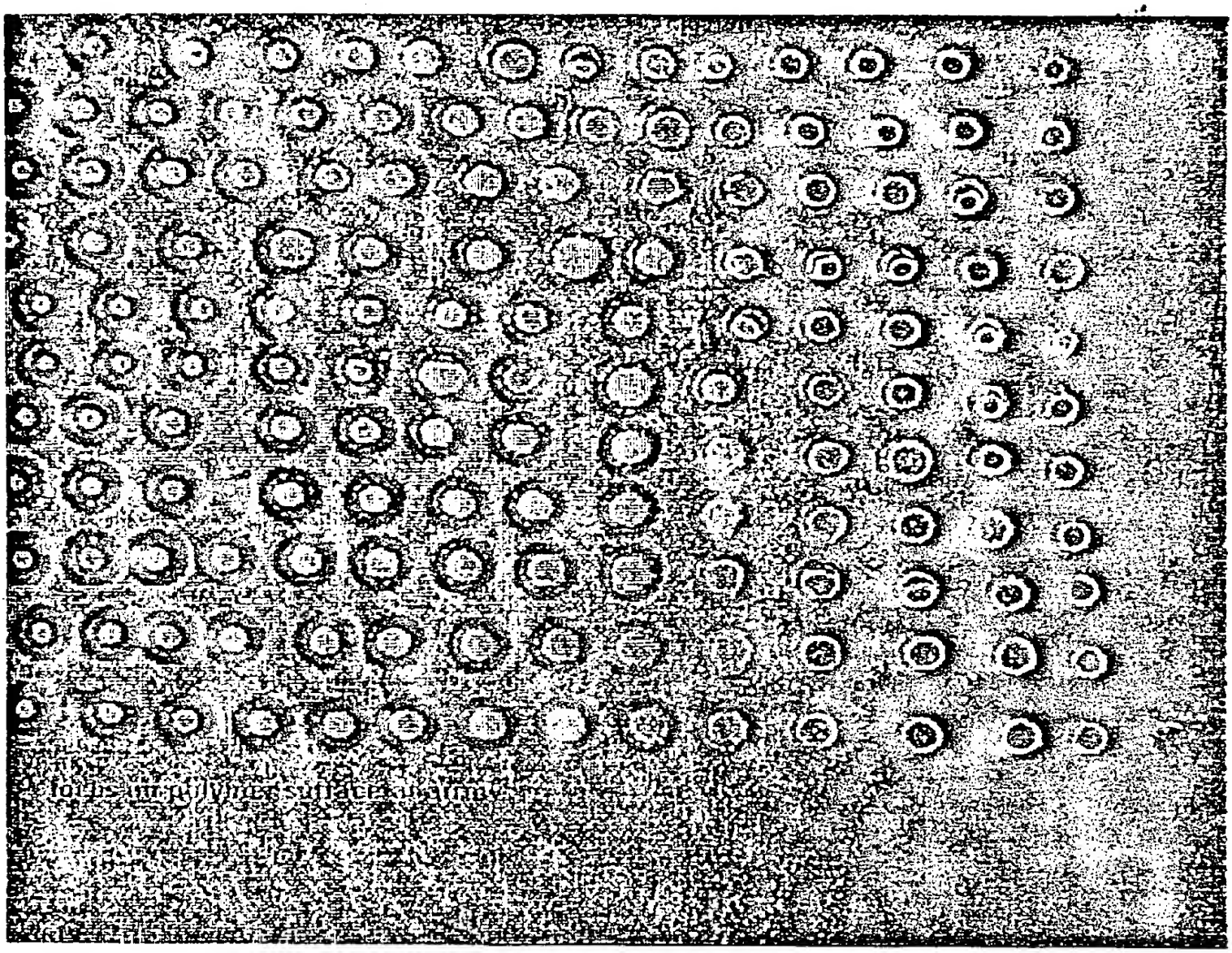
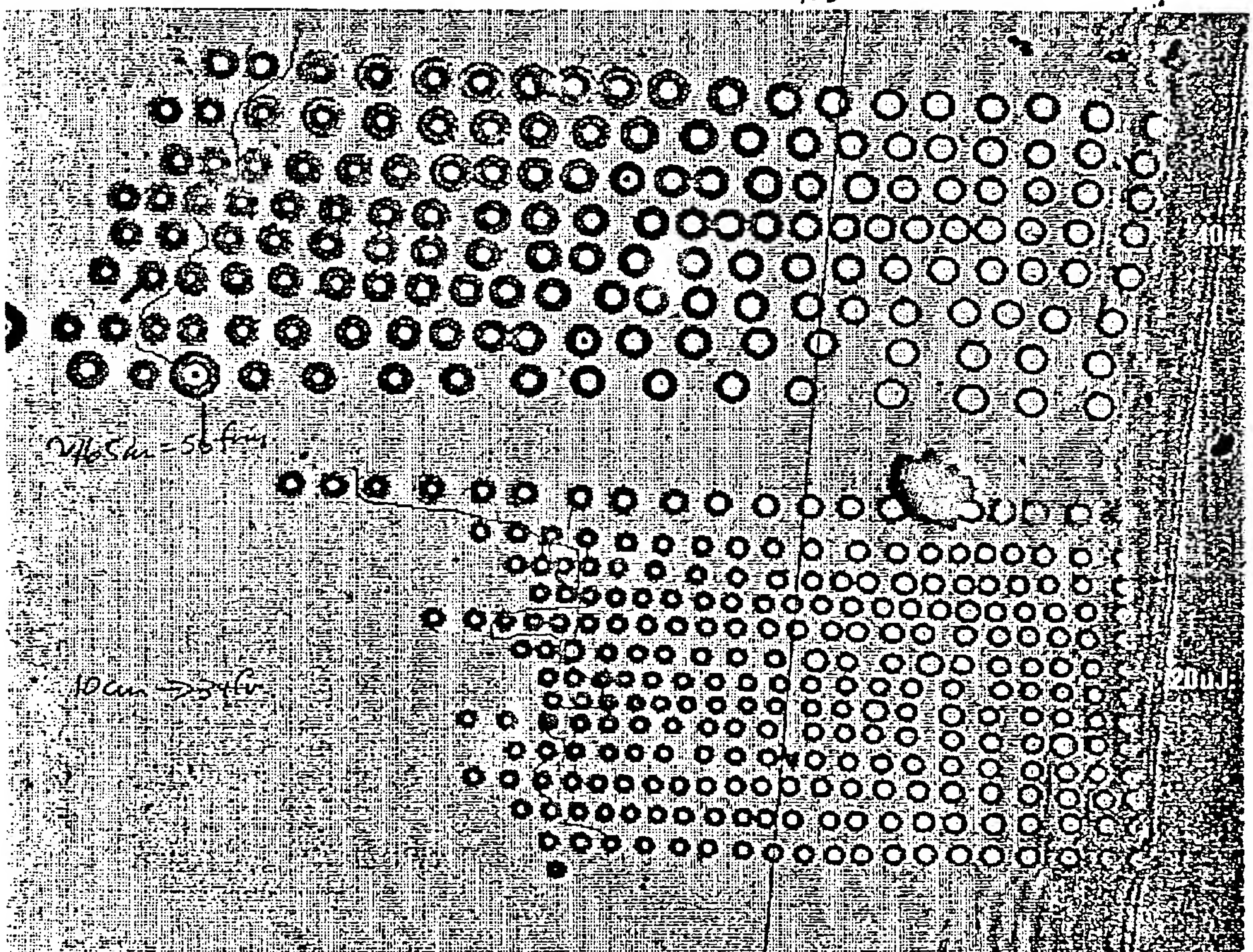


EXHIBIT 3



3.4 fines/cm
~20 fr



20.3 cm = 1.5 mm => 1 cm = 75 µm for 4x
1 cm = 30 µm for 10x = 180 µm / 6500 ~

4x -> 4 1/3 [illegible]
10x = 43 [illegible]
1 field: 1.5 m

7 fr
46 µm.

20 fr 6 cm
7 fr 17 mm